The flavour chemistry of rhubarb

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Award date:
1993

Awarding institution:
University of Bath

THE FLAVOUR CHEMISTRY

OF

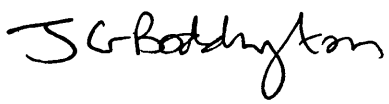
RHUBARB

Submitted by JOHN BODDINGTON

for the degree of Doctor

of Philosophy of the University of Bath

1993



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ACKNOWLEDGEMENTS

I am indebted to Barnett and Foster International for the opportunity I have been given to carry out these studies. I am especially grateful to Fiona Tinsley for the many helpful discussions throughout.

Many thanks to Mary Hardgrave at Horticulture Research International, Stockbridge House, for providing generous samples of rhubarb.

Similarly Karen Taylor must be thanked for her diligence and good humour while typing (and retyping!) this manuscript.

I acknowledge with gratitude the help and advice of Dr. Malcolm Sainsbury of The University of Bath whilst supervising these studies.

Finally I thank my parents for their encouragement and support during the many 'ups and downs' of this work.

ABSTRACT

Chapter 1 examines the botanical origins of rhubarb, discusses its taxonomical classification and briefly describes its cultivation and utilisation. A review of rhubarb literature to 1993 is also included with special reference to volatiles and volatile aglycones.

Chapter 2 presents the experimental procedures for the analysis of rhubarb; either intact, distilled, preheated or canned. Methods for quantifying rhubarb flavour volatiles are described along with those employed to observe the enzymatic hydrolysis of linoleic/linolenic acids in rhubarb.

Chapter 3 covers the extraction and concentration of rhubarb glycosides and their subsequent enzymatic hydrolysis to release volatile aglycones. The gas chromatographic and combined gas chromatographic/mass spectrometric methods of analysing the free aglycones are detailed.

Chapters 4 and 5 present the collective results of these investigations.

Chapter 6 discusses the hundred or so compounds identified in rhubarb for the first time and speculates on those volatiles present in intact rhubarb. The formation of

volatiles during homogenisation and heating is described especially in relation to sugar and amino acid degradation, carotenoid breakdown and fatty acid autoxidation. A similar process is followed for the volatiles present in canned rhubarb. The quantitation of rhubarb volatiles throughout the growing season is described and is equated to maximising flavour yields during commercial processing of rhubarb. The effect of homogenate holding time and pH on certain rhubarb volatiles is correlated and linked to the optimisation of rhubarb flavour.

Chapter 7 presents a complete review of the upwards of 113 aglycones observed in rhubarb stalk, leaf and root and compares them with those in the rest of the plant kingdom. The role of glycosides and their flavour impact in rhubarb is also discussed.

Chapter 8 provides a general conclusion to the study.

CONTENTS

CHAPTER 1

A REVIEW OF RHUBARB, ITS CULTIVATION, UTILISATION AND FLAVOUR CHEMISTRY TO 1993

The botanical origins of rhubarb

Rhubarb (*Rheum spp.*) has been described variously as originating from Manchuria, Bulgaria, Tibet and central and eastern Asia[1][2]. Initially rhubarb was cultivated for its medicinal properties, the purgative effect of the roots being described as early as 2700BC in the Chinese herbal 'Pen-ching'[3]. The dried root became a valuable export for the Chinese, and was generally transported to Western Europe *via* India and Persia[3].

The application of rhubarb root in Greek medicine is first recorded in the 1st Century A.D and such was its value to western medicine that by the mid. 17th Century it commanded a price three times that of opium.

The first seeds of *Rheum palmatum L.* were introduced to Britain in 1762 by Dr Mounsey, the chief physician to the Czar of Russia[4]. The book 'The great importance and proper method of cultivation and curing of rhubarb in Britain for medicinal uses' was written by Sir William Fordyce in 1792, coinciding with the introduction of *Rheum rhaponticum,* a species more suited to the British climate[5]. Over the next hundred years the cultivation of rhubarb spread to all European countries and subsequently to the New World. Since then inter-species hybridisation and plant breeding programmes have resulted in the development of over 200 varieties of edible rhubarb.

Classification of Rhubarb species

It is generally agreed that there are twenty-five species in the Genus *Rheum*. Those of commercial value can be separated into two categories:Chinese rhubarb and Edible rhubarb.

Chinese rhubarb

Chinese rhubarb is grown exclusively for the medicinal value of its roots and short stalks[6]. Most Chinese rhubarb is *Rheum officinale* although other workers also refer to *Rheum rhizoma*[7], *Rheum palmatum*[8] and *Rheum sinensis radix*[9].

Edible rhubarb

Edible rhubarb is grown entirely for the consumption of its stalks. *Rheum rhaponticum* is a true species growing in south east Russia and Bulgaria[7][5], whereas most modern varieties are hybrids and require root division to be propagated commercially[6]. Despite being of mixed parentage, most have *Rheum rhaponticum* as the dominant progenator[5] and as such have frequently been misquoted as this species. The rhubarb varieties in this work will therefore be referred to as *Rheum rhabarbarium* to differentiate them from the true breeding *Rheum rhaponticum* found in the wild.

Fig A.1 The taxonomy of Edible rhubarb[3]

Kingdom	:	Plantae;
Phylum	:	Spermatophyta;
Division	:	Angiospermae;
Class	:	Dicotyledoneae;
Family	:	Polygonaceae : Polygonoideae : Ruminiceae;
Genus	:	Rheum;
Species	:	rhabarbarium
Common name	:	rhubarb, pie plant
Cultivar	:	Timperly Early

Cultivation of *Rheum rhabarbarium* and its utilisation

Rheum rhabarbarium requires cold temperatures to achieve optimum growth. It does not thrive where the mean temperatures are greater than 5°C in winter and 25°C in summer[1].

Rhubarb is grown commercially in three ways:-

i) Forcing in the field under plastic.

ii) Forcing in sheds, where crowns produce stalks eight weeks earlier[6][10].

iii) Open cultivation outdoors.

Most fresh rhubarb sold in shops is forced to provide an early, high value crop. Details of propagation were outlined by The Ministry of Agriculture, Fisheries and Foods in 1984[11]. Future cultivation is likely to utilise in *vitro* techniques to allow the production of virus free plants and the multiplication of new cultivars[12].

In the most recently available figures (1986) the total area of land devoted to rhubarb cultivation in the U.K. was 950 hectares[11]. This included 100 hectares of forced rhubarb and 844 hectares of summer rhubarb (grown in the open). The total production was 31,000 tonnes with a value of £6,694,000. Apart from being canned, rhubarb constitutes an important ingredient in milk based desserts and has been employed in high value, convenience puddings and sweets. These products are often flavoured with liquidised rhubarb which presents the associated problems of seasonal supply and/or high freezing costs and variability of flavour. Currently most rhubarb flavours are nature identical and are usually based on 1-phenylethyl acetate, as yet only identified in gardenia flower oil. Clearly, further investigation of the volatile components of rhubarb, and their generation, is required to advance the development of natural and nature-identical flavours.

A Review of rhubarb literature to 1993

In 1988 D.E.Marshall wrote a Bibliography of rhubarb and

Rheum species[3] containing 3385 references from as far back as the sixteenth century. In the intervening five years a further twenty or so research papers have been published.

The majority of research has examined non-volatile constituents of rhubarb and has included the detection of oxalate[14], organic acids[14], anthocyanins and general nutritional components[15] in the stalks of *Rheum rhabarbarium*. Since the mid 1970's, workers at the Faculty of Pharmaceutical Sciences, Kyushu University, Japan, have written a series of fifteen papers entitled 'Studies on Rhubarb'[16], which have investigated the roots of several rhubarb species. To date, forty-five glycosides have been characterised of which ten were of volatile aglycones. Of these, five were found to contain 4-(4-hydroxyphenyl)-2-butanone (raspberry ketone), four cinnamyl alcohol and one cinnamic acid[8][17] linked to various sugar moieties. The Japanese workers noted that while the roots of *Rheum palmatum* and *Rheum officinale* contained many glycosides, edible rhubarbs such as *Rheum rhabarbarium* contained only stilbene glycosides at significant levels. Rhubarb stalks and leaves were not investigated.

Two papers have been directed at the volatile constituents of rhubarb species. The first concerned the occurrence of primary and secondary amines in food[18] and showed rhubarb (*Rheum rhabarbarium*) to contain ammonia (6340mg/kg), 3-methylbutylamine (3.9mg/kg), aniline (5mg/kg), benzylamine

(2.9mg/kg), phenethylamine (3.2mg/kg) and N-methyl phenethylamine (2.6mg/kg). The second looked at the volatiles present in a Freon 113 extract of Chinese rhubarb root (*Rheum sinensis radix*)[9]. The extract was separated into neutral and acid fractions prior to analysis by gas chromatography/mass spectrometry (GC/MS). Major components included naphthalene derivatives, methyl esters of higher acids and phenols. Thus it is clear that by the end of 1992 very few volatile flavour components had been identified in edible rhubarb stalk.

This study investigated in some detail the flavour volatiles present in distilled, cooked and low-temperature solvent-extracted samples of rhubarb. The volatiles associated with canned rhubarb were also elucidated in an attempt to identify possible character-impact compounds generated during processing.

The level of volatiles in a commonly grown cultivar, Steins Champagne, was observed throughout the growing season to ascertain times when the flavour content was at a maximum. Quantitation trials were also carried out to correlate whether processing time and pH could significantly affect the level of key rhubarb volatiles.

Finally, the glycoside content of the leaf, stalk and root was evaluated with respect to its possible effect on the flavour of rhubarb.

All analysis involved gas chromatography (G.C.) for separation and quantitation and combined gas chromatography/mass spectrometry (GC/MS) for tentative peak identification.

CHAPTER 2

EXPERIMENTAL PROCEDURES FOR THE ANALYSIS OF RHUBARB VOLATILES

Materials

Pure chemical standards were obtained variously from Aldrich Chemical Co, Lancaster Synthesis, Oxford Organics, International Flavours and Fragrances Ltd and Bedoukian Inc. All standards were of commercial quality being better than 98% pure.

Solvents such as methanol, dichloromethane and diethyl ether were, where possible, of H.P.L.C. grade and were redistilled directly before use. All rhubarb samples were supplied by Horticulture Research Int., Stockbridge House, Selby, N.Yorks, the exception being when fresh tissue was required for the investigation into the lipoxygenase system. These latter experiments used stalks obtained from named varieties growing locally and, for comparative studies, care was taken to ensure that plants were of the same age and were growing in the same general aspect.

Experimental procedures were developed for the following analyses:-

1) The cold dichloromethane extraction of fresh rhubarb.

2) The dichloromethane extraction of distilled rhubarb.

3) The dichloromethane extraction of preheated rhubarb.

4) The dichloromethane extraction of canned rhubarb.

5) The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles.

6) The enzymatic hydrolysis of linoleic/linolenic acids in rhubarb.

The following procedures were each repeated three times.

1 The cold dichloromethane extraction of rhubarb

Rhubarb (2000g) was homogenised for 2 minutes with water (1000g) and redistilled dichloromethane (500g) and the volatiles extracted by agitating vigorously for 20 minutes. The juice was pressed from the pulp and the dichloromethane extract separated off in a funnel. The rhubarb pulp and aqueous layer was re-extracted with dichloromethane (4x250g). The dichloromethane extractives were combined, dried over sodium sulphate (10g) and passed through a 1PS filter paper (Whatman). All operations were performed with dichloromethane at -20°C and with chilled glassware.

The dichloromethane extract was then concentrated by fractional distillation to approx. $2cm^3$ and then to $0.5cm^3$ under a nitrogen stream. Analysis was by gas chromatography linked to a mass spectrometer employing the following conditions:-

Chromatograph	Hewlett Packard 5890
Column	30m x 0.53mm i.d. vitreous silica capillary coated with DB5 (film thickness 0.25μm)
Carrier gas	Helium
Column temperature	35°C to 35°C for 3 mins, 35°C to 60°C at 2°C min^{-1}, 60°C to 220°C at 6°C min^{-1}, 220°C to 220°C for 10 mins
Carrier gas velocity	30cm sec^{-1}
Injector	Splitless: Automatic Sampler Hewlett Packard 7673A
Injector temperature	270°C
Interface	Column as above
Interface temperature	250°C
Mass spectrometer	Finnigan MAT Incos 50
Ionisation	Electron impact
Ionisation energy	70 eV
Acceleration voltage	4 kV
Focusing	Quadrupole
Detection	Electron multiplier: positive ion
Multiplier voltage	1200 V

Mass spectral identification (MS) was verified, where possible, by coelution with standard chemicals (RT) under the following analysis conditions:-

Chromatograph	AI 93

Column	30m x 0.53mm i.d. vitreous silica capillary, coated with DB5 (film thickness 0.25μm)
Column temperature	35°C to 35°C for 3 mins, 35°C to 60°C at 2°C min^{-1}, 60°C to 220°C at 6°C min^{-1}, 220°C to 220°C for 10 mins
Carrier gas	Hydrogen
Carrier gas velocity	30cm sec^{-1}
Injector (1)	Split 100:1
Injection temperature	250°C
Injection volume	1μL
Detector temperature	Flame ionisation
Detector	250°C
Recorder	Philips PM8251A linear chart recorder

Analytical results are shown in Chapter 4

2 <u>The dichloromethane extraction of distilled rhubarb</u>.

Rhubarb stalk (2000g) and water (1000g) were homogenised in a Waring blender for 2 minutes, placed in a 5L round-bottom flask and a cold-water condenser attached. Fractions (50cm^3) were gently distilled over at 5cm^3 min^{-1} with continuous stirring. Fractions were collected until aroma was no longer discernable and then two further 50cm^3

quantities were taken. The combined fractions were extracted with dichloromethane (6x50cm^3) at 4^{0}C and the solvent then dried over sodium sulphate (10g) and filtered through a 1PS filter paper (Whatman). The extract was concentrated and analysed in the same manner as the cold dichloromethane extract of rhubarb.

3 The dichloromethane extraction of preheated rhubarb

Rhubarb stalk (2000g) and water (1000g) were homogenised in a Waring blender for 2 minutes, placed in several glass bottles and sealed with crown caps. The glass bottles were placed in an oil-filled heating bath and heated under the following protocol:-

25^{0}C - 110^{0}C in 1 hr
110^{0}C - 110^{0}C for 45 mins
110^{0}C - 25^{0}C in 1 hr

The cooked pulp was then removed, extracted with solvent and analysed as for the cold dichloromethane extraction of rhubarb.

4 The dichloromethane extraction of canned rhubarb

The extraction of canned rhubarb exactly followed the

method for fresh rhubarb except canned rhubarb (2000g) (Smedley's: Hawards Foods, Scotland) was the starting material. This material included only rhubarb, rhubarb juice and water; there was no added flavouring.

5 The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles

Samples of rhubarb var. Steins Champagne were harvested on allotted days and immediately taken to the laboratory for extraction. Rhubarb stalk (333g), water (333g) and Internal Standard A (1cm^3) were homogenised for two minutes in a Waring blender. The liquidised rhubarb was squeezed through a metal sieve and the juice collected. The dry pulp remaining was re-liquidised with two further quantities of water (333g and 100g). The liquid was combined (1000-1050g yield) and extracted for 24 hrs with redistilled dichloromethane (500g) as shown in Fig.B.1.

Note:-

The dichloromethane in flask A was kept at 48°C.

Side arm B was insulated with cotton wool.

The layer of extracting dichloromethane C was carefully vortexed with rhubarb layer D, using a magnetic stirrer.

Fig.B.1

Downward displacement liquid/liquid extractor[19]

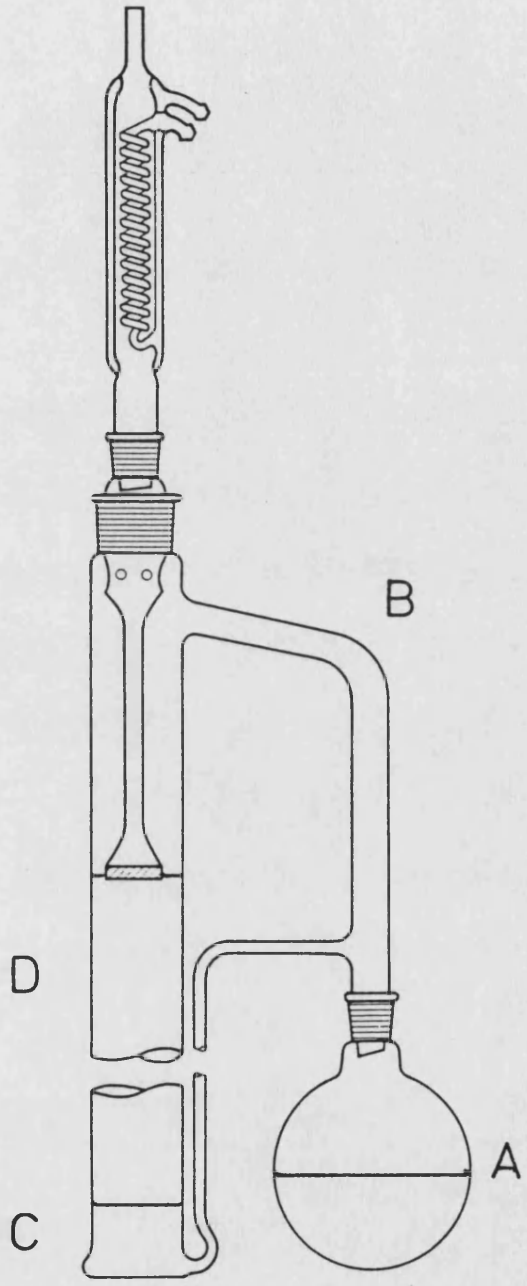


Fig.B.1

Downward displacement liquid/liquid extractor[19]

The total dichloromethane extract was dried over sodium sulphate (10g) and then passed through a 1PS filter paper (Whatman). Internal Standard B ($1cm^3$) was added and the extract carefully reduced by distillation to approx. $3cm^3$. The final stages of concentration and analysis then closely followed the protocol outlined in the cold dichloromethane extraction of rhubarb.

Composition of Internal Standard A:-
0.1% m/v Octan-2-ol in ethanol. This allowed a degree of correction for the non-100% extraction of rhubarb volatiles.

Composition of Internal Standard B:-
0.04% m/v Amyl dodecanoate, 0.04% m/v Heptan-4-one in dichloromethane. This allowed correction for any variation in gas chromatography.

6 The enzymatic hydrolysis of linoleic/linolenic acids in rhubarb

Method 1: The effect of homogenate standing

All trials were carried out on freshly picked stalks of rhubarb (var. Steins Champagne).

Rhubarb stalk (100g) was chopped into water (100g) and

liquidised for 60 seconds in a Waring blender. Homogenates were allowed to stand for periods of 0, 30, 60 and 120 minutes at 18°C. Enzymatic processes were then inhibited by homogenisation with methanol (66g) for 30 seconds. Internal Standard A (1cm^3) was also added at this point. The rhubarb homogenates were extracted with dichloromethane (4 x 100g) and the combined dichloromethane extractives dried over sodium sulphate (5g) and passed through a 1PS filter paper (Whatman). A Kuderna-Danish apparatus was used to concentrate the dichloromethane extract to approx. 3cm^3. The extract was further reduced to 1cm^3 under nitrogen and then analysed by GC using the following conditions:-

Chromatograph	AI 93
Column	30m x 0.53mm i.d. vitreous silica capillary, coated with DB5 (film thickness 0.25μm)
Column temperature	35°C to 35°C for 3 mins, 35°C to 60°C at 2°C min^{-1}, 60°C to 220°C at 6°C min^{-1}
Carrier gas	Hydrogen
Carrier gas velocity	30cm sec^{-1}
Injector	Split 100:1
Injection temperature	250°C
Injection volume	0.2 μl
Detector	Flame ionisation

Recorder	Philips PM8251A linear chart recorder

Volatiles were quantified by comparison of peak areas with those of standard chemicals.

Method 2 : Effect of homogenate pH.

As for method 1 except:-

i) Frozen and thawed rhubarb stalk was used.

ii) Rhubarb was added to phosphate/citric acid buffer adjusted to different pH values.

iii) Enzymes were inhibited at time:90 minutes, except the control which was inhibited at time:0 minutes.

CHAPTER 3

EXPERIMENTAL PROCEDURES FOR THE ANALYSIS OF RHUBARB GLYCOSIDES

MATERIALS

Chemical standards, solvents and rhubarb samples were obtained and treated as outlined in Chapter 2. Rhubarb leaves were collected from a locally grown variety during June whereas rhubarb roots were collected from dormant plants in November and December.

The glycosidic enzymes employed in this work, β-glucosidase and Macer8 FJ, were obtained from Biocatalyst Ltd and were stored and used as directed by the supplier.

GLYCOSIDES IN RHUBARB

A method was adapted from that of Gunata *et al.*[20] which enabled separation of glycosides from solids, pectic substances and non-bound volatiles. Each analysis was repeated three times.

SAMPLE PREPARATION

Rhubarb material (1000g) was added to 0.2M Na_2HPO_4/NaH_2PO_4 buffer (250g) maintained to a pH of 8, and liquidised. The juice was pressed from the pulp and then centrifuged at 4000 rpm for 30 minutes. Sulphur dioxide was added as a stabiliser. All operations were performed at 4°C.

COLUMN PREPARATION

Amberlite XAD-2 [BDH Chemicals 20-50 mesh] was suspended in methanol and poured, to a depth of 15cm, into a glass column (30x 1cm i.d.) fitted with a P.T.F.E tap. The column was washed with methanol (75cm^3), diethyl ether (75cm^3) and distilled water (75cm^3).

FRACTIONATION OF SAMPLE

All samples and solvents were passed through the column at a flow rate of 2-3 cm^3 min^{-1}. The juice collected from the rhubarb matter was applied to the column, which was then rinsed with water (100cm^3) to remove sugars, acids and other water-soluble compounds. Free volatiles fixed on the column were eluted using pentane (100 cm^3). The glycosides were washed from the column with methanol (100 cm^3), dried over sodium sulphate (1g) and filtered (Whatman filter paper No. 4). The methanol extract was then evaporated to dryness at 50°C leaving a residue of glycosides.

HYDROLYSIS OF GLYCOSIDES

The glycoside residue was redissolved with 0.2M citric acid/NaH_2PO_4 buffer (25 cm^3) at pH 5.7 and extracted with diethyl ether (5 x 10cm^3). β-Glycosidase (300μg) and Macer8 FJ (300μg) (Biocatalysts Ltd) were added to the buffer layer to hydrolyse the glycosides. Hydrolysis occurred in

a sealed vessel at 40°C for 48 hrs. Released aglycones were extracted exhaustively with diethyl ether [5x10cm^3]. The combined diethyl ether extracts were concentrated to 0.2 cm^3 by careful distillation and then reduced further under a stream of nitrogen. A blank trial, without rhubarb matter, was carried out to identify artifacts or contaminants. All extracts were stored at minus 25°C until they were analysed. Analysis was by gas chromatography linked to a mass spectrometer. Separation was carried out on two capillary columns (DB-5, DB-WAX) using the following conditions:-

1) DB-5 Capillary Column

Chromatograph	Hewlett Packard 5890
Column	30 m x 0.53 mm i.d. vitreous silica capillary coated with DB5 (film thickness 0.25μm)
Carrier gas	Helium
Column temperature	35°C to 35°C for 3 mins, 35°C to 60°C at 2°C min^{-1}, 60°C to 220°C at 6°C min^{-1}, 220°C to 220°C for 10 mins
Carrier gas velocity	30 cm sec^{-1}
Injector	Splitless: Automatic Sampler Hewlett Packard 7673A
Injector temperature	270°C
Interface	Column as above

Interface temperature	250°C
Mass spectrometer	Finnigan MAT Incos 50
Ionisation	Electron Impact
Ionisation energy	70 eV
Acceleration voltage	4 kV
Focusing	Quadrupole
Detection	Electron multiplier: positive ion
Multiplier voltage	1200V

2) DB-WAX Capillary Column

Chromatograph	Hewlett Packard 5890
Column	30m x 0.53 mm i.d. vitreous silica capillary coated with DB-WAX (film thickness 0.5μm)
Carrier gas	Helium
Column temperature	35°C to 35°C for 3 mins, 35°C to 60°C at 2°C min^{-1}, 60°C to 200°C at 6°C min^{-1}, 200°C to 200°C for 40 mins
Carrier gas velocity	30cm sec^{-1}
Injector	Splitless: Automatic Sampler Hewlett Packard 7673A
Injector temperature	200°C
Interface	Column as above

Interface Temperature	200°C
Mass Spectrometer	Finnigan MAT Incos 50
Ionisation	Electron Impact
Ionisation energy	70 eV
Acceleration voltage	4 kV
Focusing	Quadrupole
Detection	Electron multiplier: positive ion
Multiplier voltage	1140V

Mass spectral identifications (MS) were confirmed, where possible, by coelution with standard chemicals (RT) - see results tables: Chapter 5.

CHAPTER 4

ANALYTICAL RESULTS

RHUBARB VOLATILES

Results obtained for the analyses were as follows:-

1) The dichloromethane extraction of fresh rhubarb

Chromatogram : Fig.D.1

Compounds identified : Table IV.1

2) The dichloromethane extraction of distilled rhubarb

Chromatogram : Fig.D.2

Compounds identified : Table IV.2

3) The dichloromethane extraction of preheated rhubarb

Chromatogram : Fig.D.3

Compounds identified : Table IV.3

4) The dichloromethane extraction of canned rhubarb

Chromatogram : Fig.D.4

Compounds identified : Table IV.4

5) The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles

Chromatogram : Fig.D.5

Compounds quantified : Table IV.5

6) The enzymatic hydrolysis of linoleic/linolenic acids in rhubarb

Volatile formation : Fig.D.6, Table IV.6

pH effect: Fig.D.7, Table IV.7

In all tables peak identifications are labelled MS and RT. MS indicates a positive correlation between an unknown compound's ion fragmentation spectra and that of a standard chemical, or of a library record of that chemical. The libraries employed were i) NBS/EPA/NIH library and ii) FO library [Finnigan Corp].

The Incos 50 reduces unknowns to the 16 largest peaks and compares these with libraries using three search strategies: i) PURITY, which measures the resemblance of the unknown to the library, ii) FIT, which measures the degree to which the library spectra occurs in the unknown and iii) REVERSE FIT, which determines the degree of unknown present in the library. In practice, FIT is generally of most use and in this study a FIT greater than 850 was considered as a possible, and one exceeding 950 a probable, identification.

RT indicates that the component coelutes with an authentic sample of the compound. In many cases however, retention times were found to vary by several seconds over an entire run. Therefore, whenever internal standards and known major peaks differed between runs, the retention times of subsequently eluting unknowns were also corrected. In this way it was possible to define coelution as occurring within +/-2 seconds of that of an authentic compound.

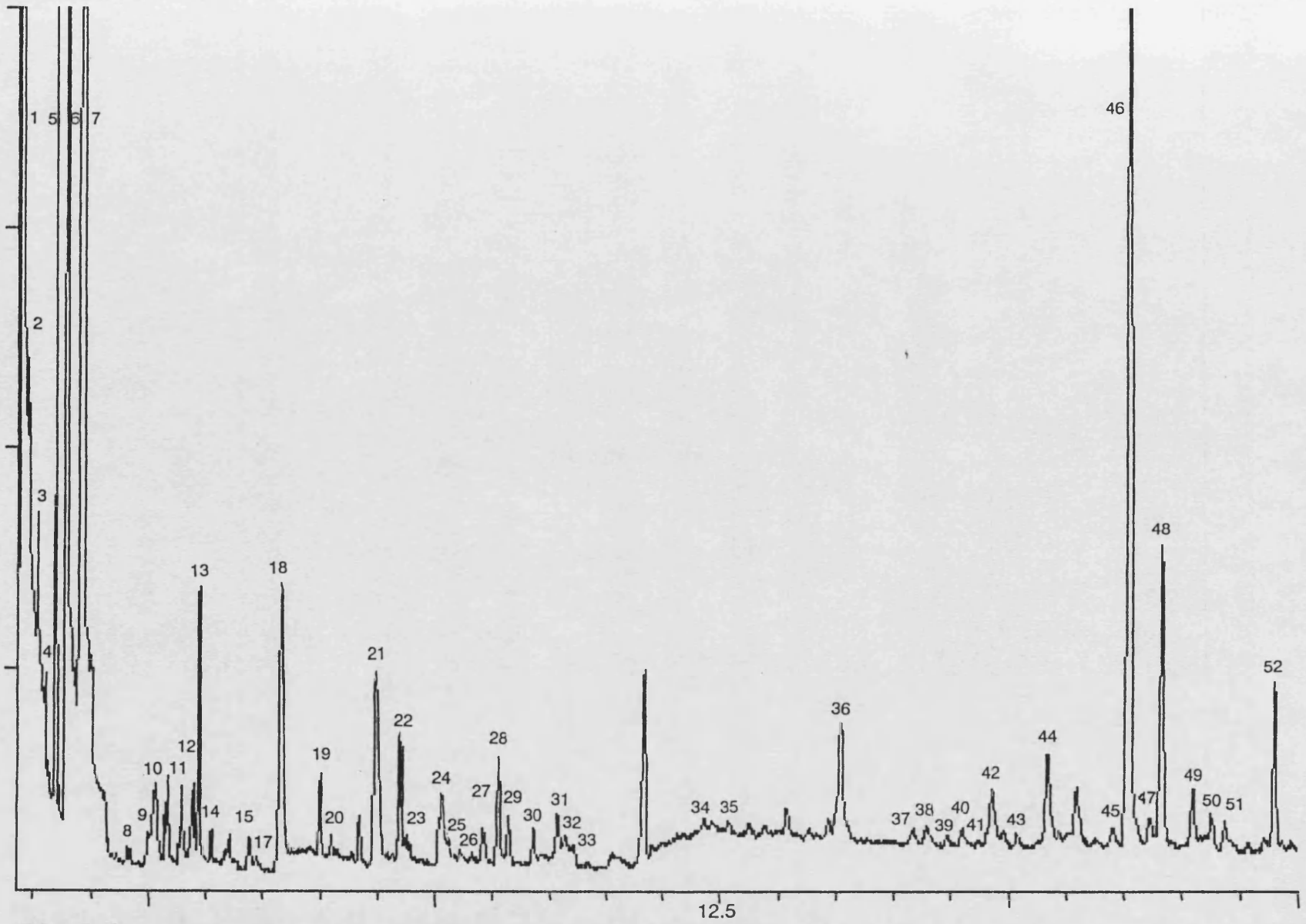
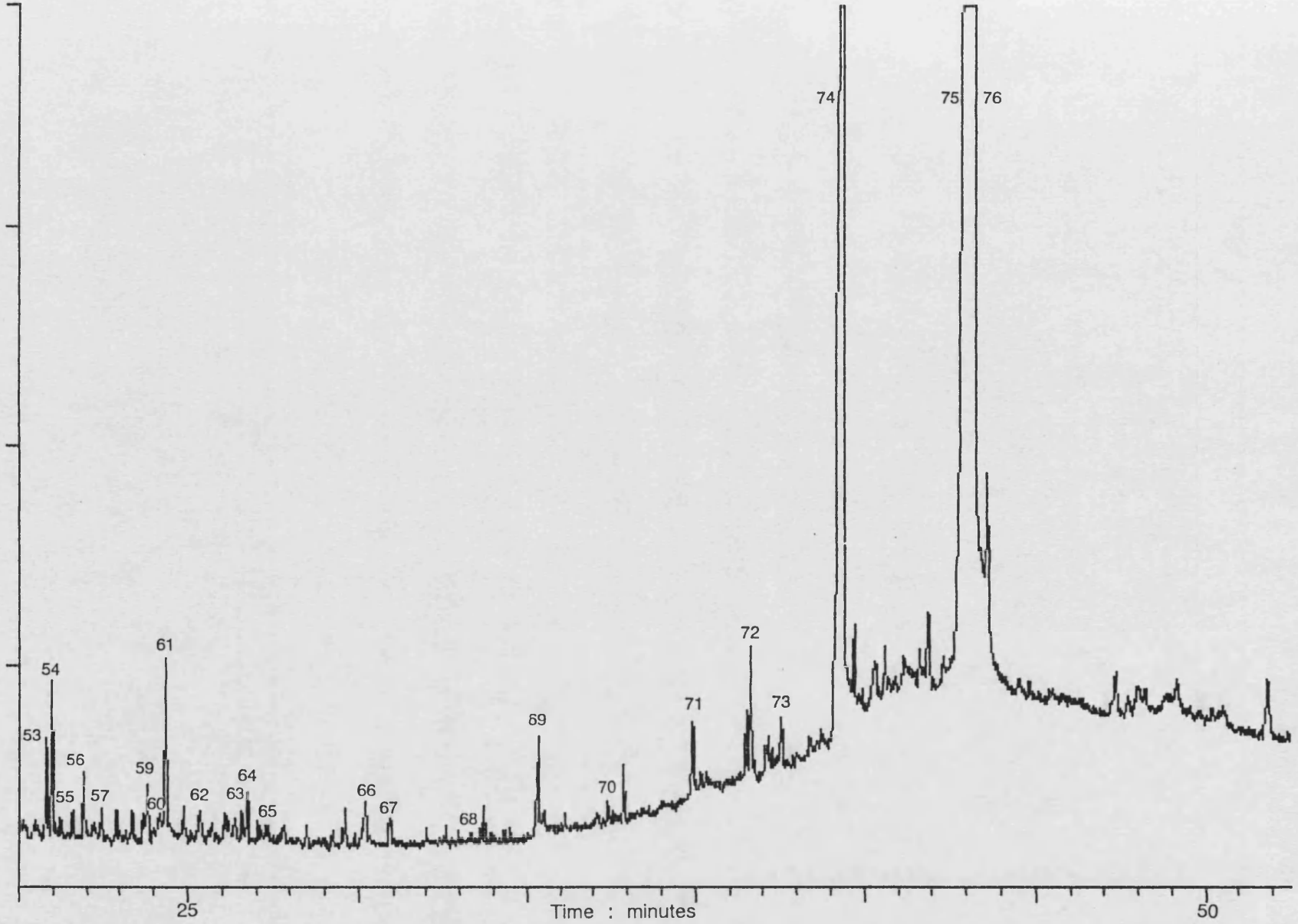


Fig.D.1 GC trace of the cold dichloromethane extractive of fresh rhubarb stalk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65
70
68
25
Time : minutes
50

Table IV.1 Peak identification : Fresh rhubarb

1	2-Methyl-3-buten-2-ol	MS RT
2	Ethyl acetate	MS RT
3	2-Methyl-2-butanol	MS RT
4	2-Butenal	MS
5	Butanol	MS RT
6	3-Penten-2-one	MS RT
7	3-Penten-2-ol	MS RT
8	Methyl butyrate	MS RT
9	3-Methybutanol/2-Methylbutanol	MS RT
10	2-Methyl-2-butenal	MS
11	Unknown. 85m/z base peak	MS
12	Octane	MS RT
13	Toluene	MS RT
14	4-Hexen-3-one	MS
15	3-Methyl-2-butenol	MS RT
16	Hexanal	MS RT
17	Unknown	
18	*cis*-3-Hexenal	MS RT
19	Unknown. 85m/z base peak	MS
20	Butyl acetate	MS RT
21	Acetal of 3-penten-2-one (tent.)	MS
22	4-Methylpentanol	MS
23	Diacetone	MS RT
24	*trans*-2-Hexenal	MS RT
25	1,4-Dimethylbenzene	MS RT
26	*cis*-3-Hexenol	MS RT

27	1,3-Dimethylbenzene	MS RT
28	*trans*-2-Hexenol	MS RT
29	Hexanol	MS RT
30	3-Methylbutyl acetate	MS RT
31	Styrene	MS RT
32	1,2-Dimethylbenzene	MS RT
33	2-Methylbutyric acid	MS RT
34	Methyl hexanoate	MS RT
35	α-Pinene	MS RT
36	Unknown	
37	Sabinene	MS RT
38	Unknown	
39	Octen-3-one	MS RT
40	Octen-3-ol	MS RT
41	Phenol	MS RT
42	Decane	MS RT
43	*trans,trans*-2,4-heptadienal	MS
44	Octanal	MS RT
45	*p*-Cymene	MS RT
46	Limonene	MS RT
47	Benzyl alcohol/2-Ethylhexanol	MS RT
48	Salicylaldehyde	MS RT
49	Unknown	
50	Unknown	
51	γ-Terpinene	MS RT
52	Octanol	MS RT
53	Linalool	MS RT
54	Nonanal	MS RT

55	2-Phenylethanol	MS RT
56	Unknown	
57	Camphor	MS RT
58	Menthol	MS RT
59	Terpinen-4-ol	MS RT
60	Benzoic acid	MS RT
61	Isomer of carveol	MS
62	Isomer of carvone	MS
63	*p*-Anisaldehyde	MS RT
64	4-Allylphenol	MS RT
65	Nonanoic acid	MS RT
66	Decanoic acid	MS RT
67	Vanillin	MS RT
68	β-Ionone	MS RT
69	Dodecanoic acid	MS RT
70	Unknown sesquiterpene	MS
71	Tetradecanoic acid	MS RT
72	Guaiol	MS
73	Unknown	
74	Hexadecanoic acid	MS RT
75	Linoleic acid	MS RT
76	Linolenic acid	MS RT

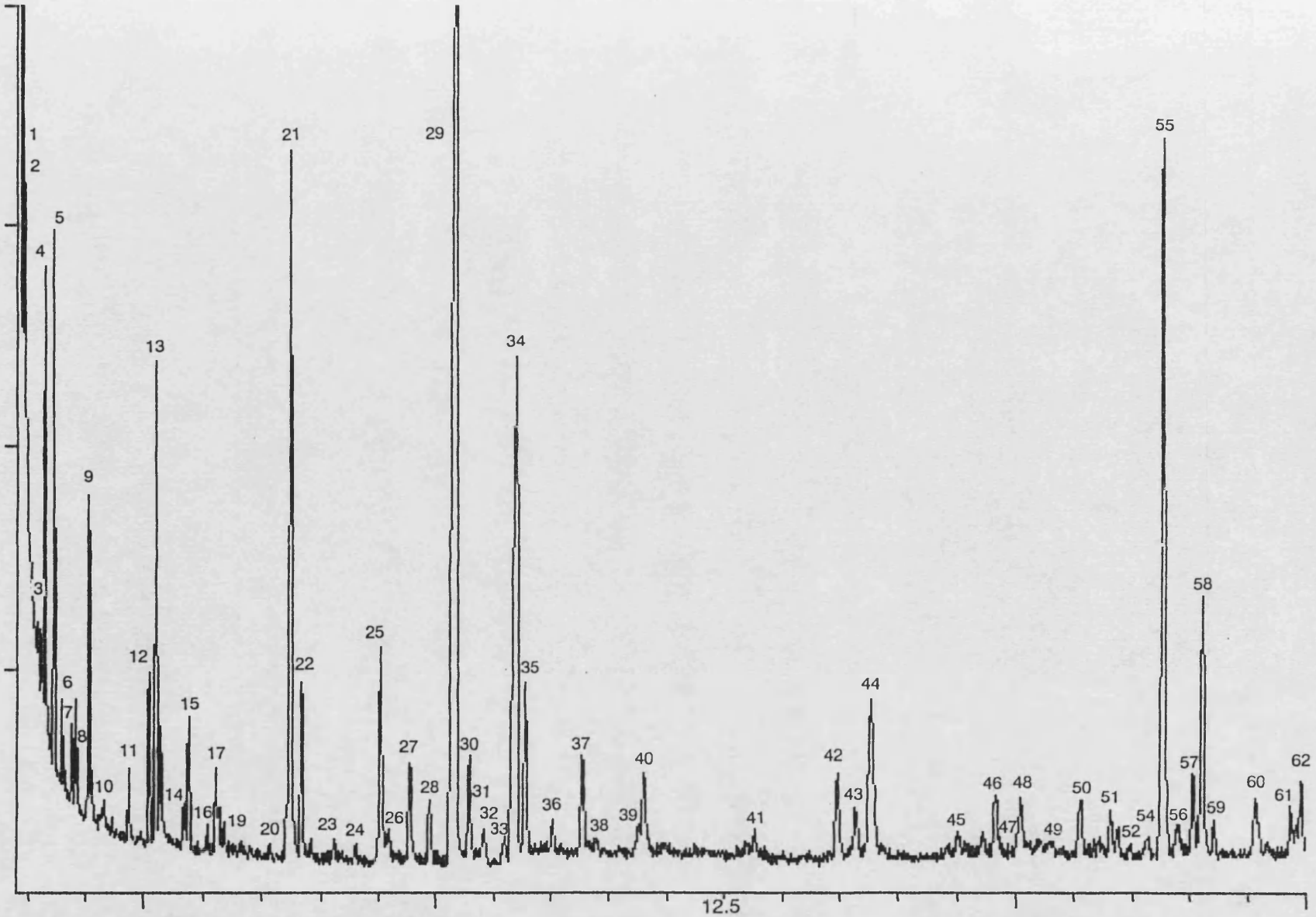
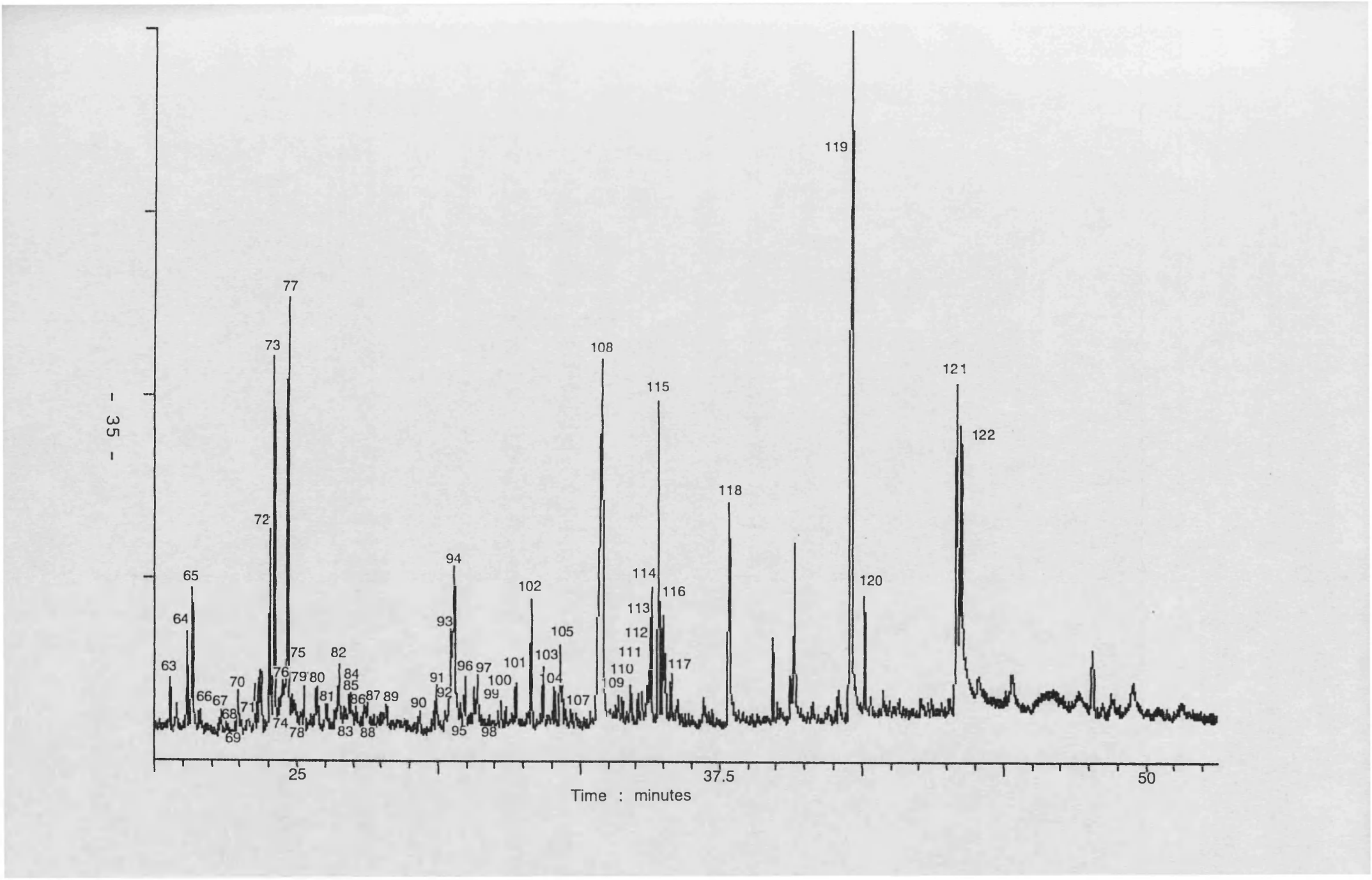


Fig.D.2 GC trace of the dichloromethane extractive of distilled rhubarb stalk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69
25
37.5
50
Time : minutes

Table IV.2 Peak identification : Distilled rhubarb

1	Ethyl acetate	MS RT
2	Unknown	
3	2-Butenal	MS
4	3-Methylbutanal	MS RT
5	2-Methylbutanal	MS RT
6	3-Penten-2-one	MS RT
7	Acetic acid	MS RT
8	Unknown	
9	Pentanal	MS RT
10	Unknown. 85m/z base peak	MS
11	Methyl butyrate	MS RT
12	3-Methylbutanol	MS RT
13	2-Methylbutanol/2-Methyl-2-butenal	MS RT
14	*trans*-2-Pentenal	MS RT
15	Pyrrole	MS RT
16	Toluene	MS RT
17	Pentanol	MS RT
18	4-Hexen-3-one	MS
19	*cis*-2-Pentenol	MS RT
20	Unknown	
21	Hexanal	MS RT
22	Ethyl butyrate	MS RT
23	Unknown. 85m/z base peak	MS
24	Butyl acetate	MS RT
25	Furfural	MS RT
26	Unknown	

27	4-Methylpentanol	MS
28	*trans*-3-Hexenal	MS RT
29	*trans*-2-Hexenal	MS RT
30	*cis*-3-Hexenol	MS RT
31	1,4-Dimethylbenzene	MS RT
32	2-Methyl-3-pentenol/Unknown	MS RT
33	1,3-Dimethylbenzene	MS RT
34	*trans*-2-Hexenol	MS RT
35	Hexanol	MS RT
36	3-Methylbutyl acetate	MS RT
37	Styrene	MS RT
38	2-Heptanone	MS RT
39	Heptanal	MS RT
40	Methional	MS RT
41	Methyl hexanoate	MS RT
42	4-Methylhexanol	MS
43	Ethyl 3-oxobutyrate	MS RT
44	*trans*-2-Heptenal/Benzaldehyde	MS RT
45	Heptanol	MS RT
46	Octen-3-one	MS RT
47	Octen-3-ol	MS RT
48	6-Methyl-5-hepten-2-one/Phenol	MS RT
49	Hexanoic acid	MS RT
50	Ethyl hexanoate/Octanal	MS RT
51	1,4-Cineol	MS RT
52	Hexyl acetate	MS RT
53	Unknown	
54	*p*-Cymene	MS RT

55	Limonene	MS RT
56	2-Ethylhexanol	MS RT
57	Salicylaldehyde	MS RT
58	Phenylacetaldehyde	MS RT
59	Unknown	
60	*trans*-2-Octenal/γ-Terpinene	MS RT
61	*cis*-2-Octenol	MS RT
62	Octanol/*trans*-Linalool-oxide (furan)	MS RT
63	*cis*-Linalool-oxide (furan)	MS RT
64	Linalool	MS RT
65	Nonanal	MS RT
66	2-Phenylethanol	MS RT
67	Unknown	
68	Terpineol	MS RT
69	Camphor	MS RT
70	β-Terpineol	MS RT
71	*trans*-2-Nonenal	MS RT
72	Menthol	MS RT
73	Terpinen-4-ol	MS RT
74	*p*-Methylacetophenone	MS RT
75	Octanoic acid	MS RT
76	Cymene-8-ol	MS
77	α-Terpineol	MS RT
78	2-(2-Butoxyethoxy)ethanol	MS RT
79	Decanal	MS RT
80	Benzothiazole/Isomer of carveol	MS RT
81	Unknown terpene	MS
82	Isomer of carvone	MS

83	Unknown	
84	*p*-Anisaldehyde	MS RT
85	4-Allylphenol	MS RT
86	*trans*-2-Decenal	MS RT
87	Geranial	MS RT
88	Nonanoic acid	MS RT
89	Menthyl acetate	MS RT
90	Triacetin	MS
91	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid ester (1-hydroxy unbound)	MS RT
92	Geranyl acetate	MS RT
93	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid ester (3-hydroxy unbound)	MS RT
94	Decanoic acid	MS RT
95	Unknown	
96	Unknown sesquiterpene	MS RT
97	Eugenol methyl ether	MS RT
98	α-Ionone	MS RT
99	Unknown norisoprenoid 190m/z base peak	MS
100	Unknown sesquiterpene	MS
101	Geranylacetone	MS RT
102	Unknown	
103	β-Ionone	MS RT
104	Pentadecane	MS RT
105	Butylated hydroxytoluene (B.H.T.)	MS RT
106	Unknown	
107	Dihydroactinidiolide/Unknown	MS RT
108	Dodecanoic acid	MS RT

109	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid diester	MS RT
110	Unknown sesquiterpene alcohol	MS
111	Guaiol	MS
112	Unknown	
113	Unknown	
114	Unknown sesquiterpene alcohol	MS
115	Unknown sesquiterpene	MS
116	Unknown sesquiterpene	MS
117	Heptadecane	MS RT
118	Tetradecanoic acid	MS RT
119	Hexadecanoic acid	MS RT
120	Unknown	
121	Linoleic acid	MS RT
122	Linolenic acid	MS RT

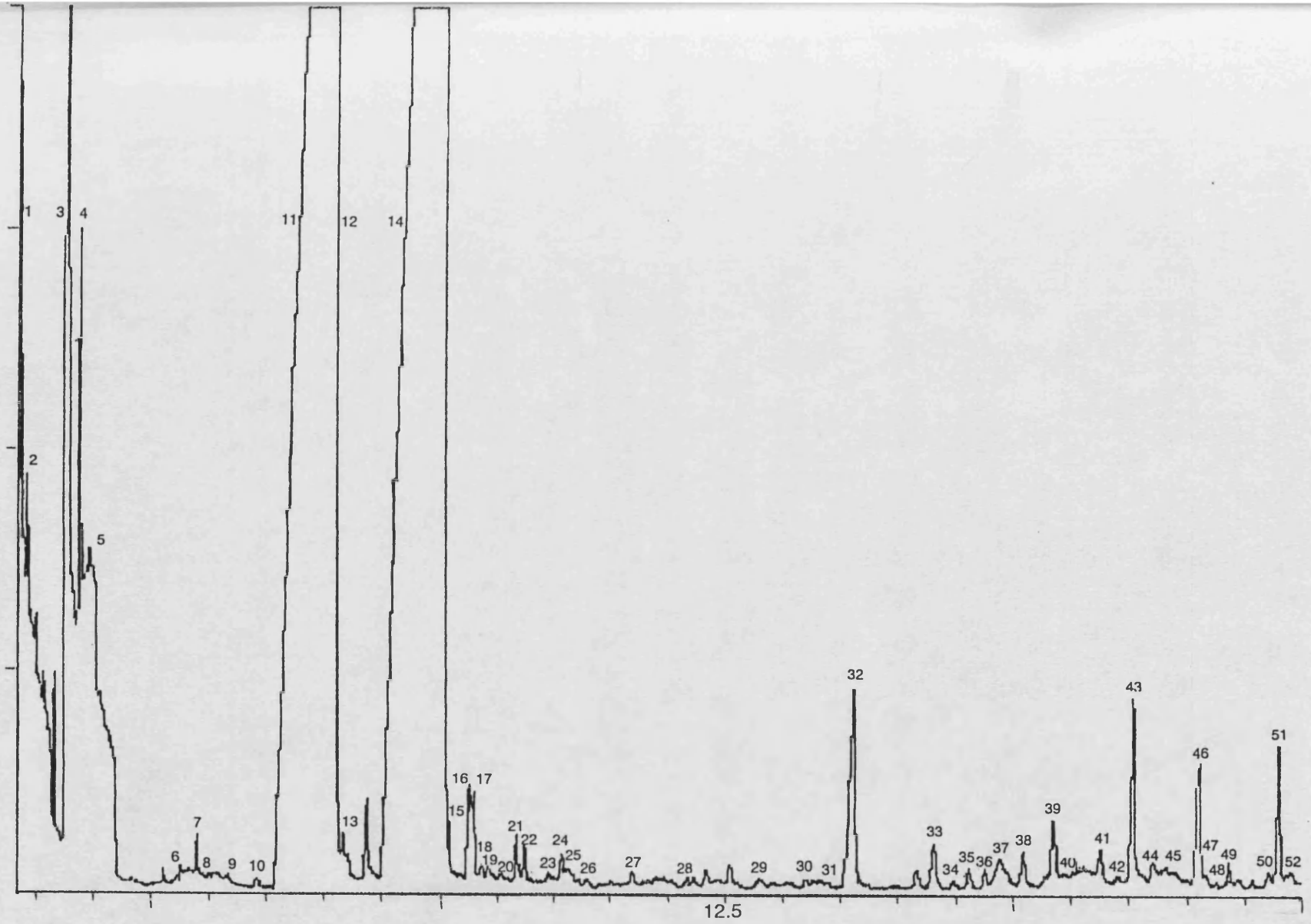
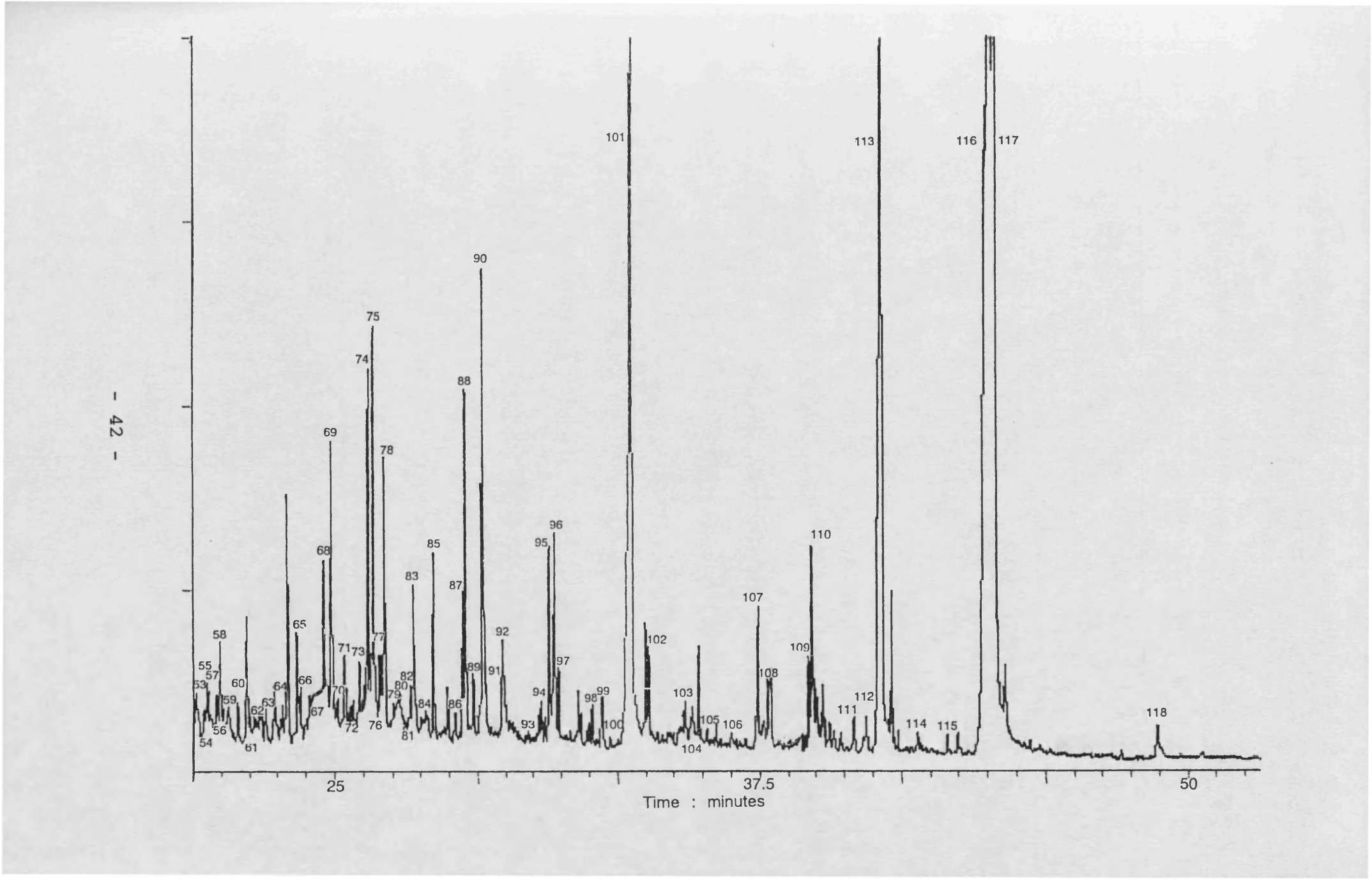


Fig.D.3 GC trace of the dichloromethane extractive of preheated rhubarb stalk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25
37.5
50
Time : minutes

Table IV.3 Peak identification : Preheated rhubarb

1	2-Methyl-3-buten-2-ol	MS RT
2	Ethyl acetate	MS RT
3	3-Penten-2-one	MS RT
4	3-Penten-2-ol	MS RT
5	Acetic acid	MS RT
6	Unknown. 85m/z base peak	MS
7	Toluene	MS RT
8	4-Hexen-3-one	MS RT
9	Unknown	
10	Unknown	
11	Hexanal	MS RT
12	*cis*-3-Hexenal	MS RT
13	Unknown. 85m/z base peak	MS
14	Acetal of 3-penten-2-one (tent.)	MS
15	Unknown	
16	*trans*-2-Hexenal	MS RT
17	Unknown	
18	*cis*-3-Hexenol	MS RT
19	1,3-Dimethylbenzene	MS RT
20	Unknown	
21	*trans*-2-Hexenol	MS RT
22	Hexanol	MS RT
23	3-Methylbutyl acetate	MS RT
24	Styrene	MS RT
25	Cyclohexanone	MS RT
26	2-Heptanone	MS RT

27	C_5 γ-Lactone.m/z 85 base peak	MS
28	Unknown	
29	Unknown	
30	*cis*-2-Heptenal	MS RT
31	4-Methylhexanol	MS
32	*trans*-2-Heptenal/Benzaldehyde	MS RT
33	Heptanol	MS RT
34	Octen-3-one	MS RT
35	Octen-3-ol	MS RT
36	Unknown	
37	6-Methyl-5-hepten-2-one/Phenol	MS RT
38	*trans,trans*-2,4-Heptadienal	MS RT
39	Octanal	MS RT
40	Hexanoic acid	MS RT
41	1-Methoxy-3-Methylbenzene	MS
42	*p*-Cymene	MS RT
43	Limonene	MS RT
44	2-Ethylhexanol	MS RT
45	Salicylaldehyde	MS RT
46	Phenylacetaldehyde	MS RT
47	Unknown	
48	γ-Terpinene	MS RT
49	*trans*-2-Octenal	MS RT
50	*cis*-2-Octenol	MS RT
51	Octanol/*trans*-Linalool-oxide (furan)	MS RT
52	Methyl 3-furoate	MS
53	*cis*-Linalool-oxide (furan)	MS RT
54	Heptanoic acid	MS RT

55	Unknown	
56	Linalool	MS RT
57	C_{11} Alkane	MS RT
58	Nonanal	MS RT
59	2-Phenylethanol	MS RT
60	Fenchol	MS RT
61	Unknown	
62	Terpineol	MS RT
63	β-Terpineol	MS RT
64	*trans*-2-Nonenal	MS RT
65	Menthol	MS RT
66	Naphthalene/Terpinen-4-ol	MS RT
67	α-Terpineol	MS RT
68	Octanoic acid	MS RT
69	2-(2-Butoxyethoxy)ethanol	MS RT
70	Decanal	MS RT
71	Benzothiazole	MS RT
72	Unknown	
73	5-Hydroxymethyl-2-furfural	MS RT
74	Unknown	
75	Unknown	
76	*p*-Anisaldehyde	MS RT
77	4-Allylphenol	MS RT
78	*trans*-2-Decenal	MS RT
79	Decanol	MS RT
80	Nonanoic acid	MS RT
81	*d*-Nonalactone	MS RT
82	Unknown	

83	Menthyl acetate/*trans,cis*-2,4-Decadienal	MS RT
84	Unknown sequiterpene	MS
85	*trans,trans*-2,4-Decadienal	MS RT
86	Unknown	MS RT
87	Triacetin	MS
88	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid ester (1-hydroxy unbound)	MS RT
89	*trans*-2-Undecenal	MS RT
90	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid ester (3-hydroxy unbound)	MS RT
91	Decanoic acid	MS RT
92	Vanillin	MS RT
93	*trans*-Cinnamic acid/Norisoprenoid 190m/z	MS RT
94	Unknown	
95	Unknown	
96	γ-Decalactone	MS RT
97	β-Ionone/Unknown	MS RT
98	Pentadecane	MS RT
99	Dihydroactinidiolide	MS RT
100	2-Methylpropyl decanoate	MS RT
101	Dodecanoic acid	MS RT
102	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid diester	MS RT
103	Guaiol	MS
104	Syringaldehyde	MS RT
105	Heptadecane	MS RT
106	Methyl gallate	MS
107	Tetradecanoic acid	MS RT

108	Hexyl decanoate	MS RT
109	6,10,14-Trimethyl-2-pentadecanone	MS
110	Caffeine	MS
111	Unknown norisoprenoid	MS
112	2-Methylpropyl tetradecanoate	MS RT
113	Hexadecanoic acid	MS RT
114	Unknown	
115	Unknown alkane	
116	Linoleic acid	MS RT
117	Linolenic acid	MS RT
118	Chrysophanic acid	MS

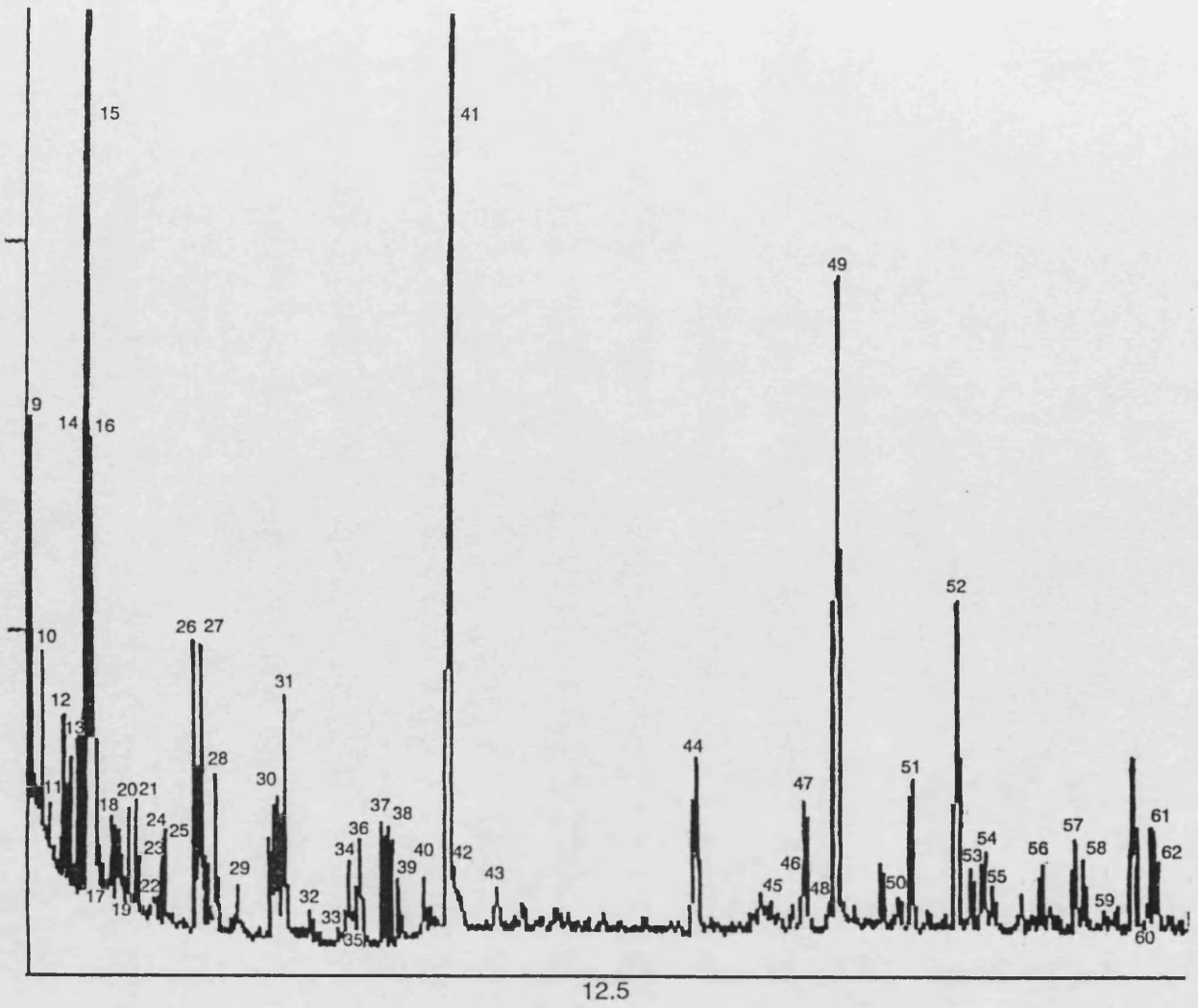
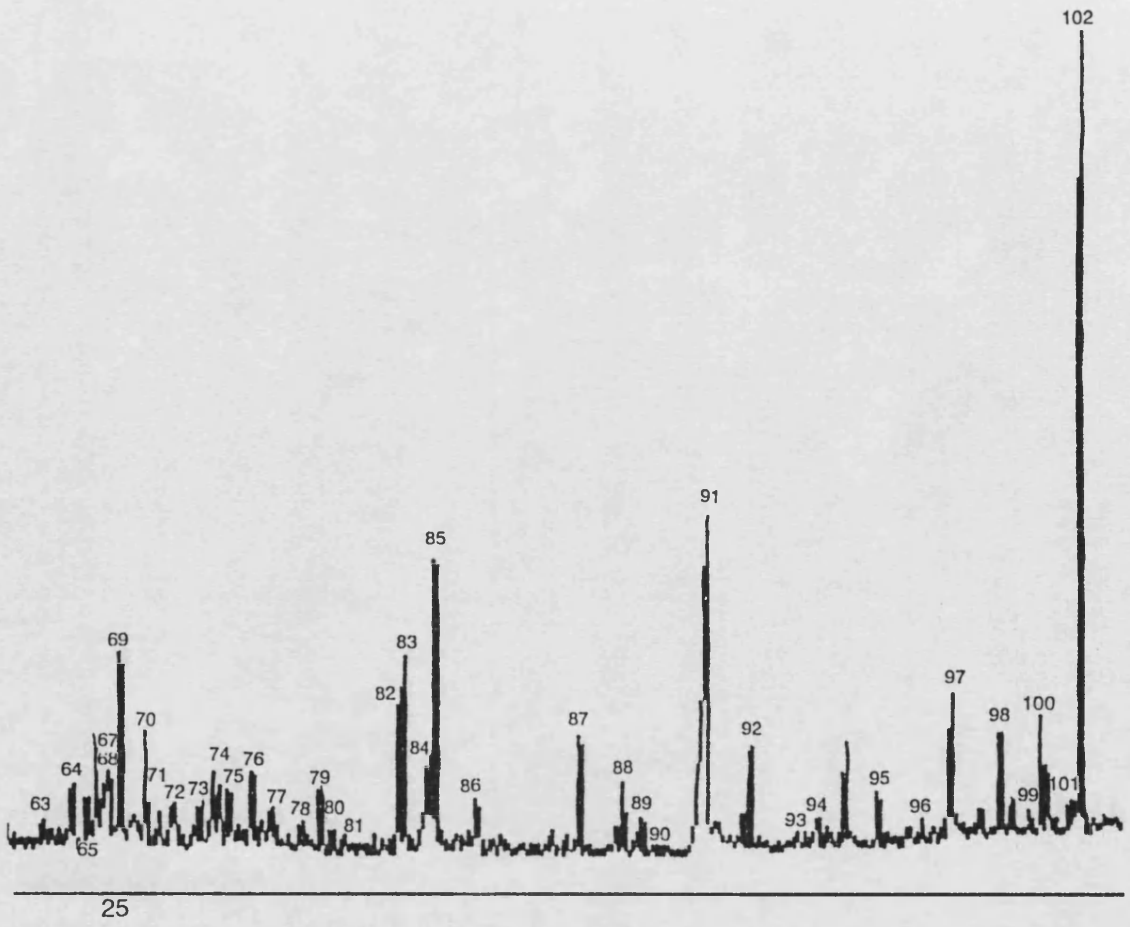


Fig.D.4 GC trace of the dichloromethane extractive of canned rhubarb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99
101
81
90
65
25
Time : minutes

Table IV.4 Peak identification : Canned rhubarb

1	2-Butanone	MS RT
2	Ethyl acetate	MS RT
3	3-Methylbutanol	MS RT
4	3-Methyl-2-butanone	MS RT
5	Butanol	MS RT
6	3-Penten-2-one	MS RT
7	3-Penten-2-ol	MS RT
8	Acetic acid	MS RT
9	3-Pentanone	MS RT
10	Acetoin	MS RT
11	Unknown	
12	Methyl butyrate	MS RT
13	Unknown	
14	3-Methylbutanol	MS RT
15	Pyridine	MS RT
16	Propylene glycol	MS RT
17	2-Methyl-3-pentanone	MS RT
18	Unknown. 85m/z base peak	MS
19	Octane	MS RT
20	Toluene	MS RT
21	Pentanol	MS RT
22	*cis*-2-Pentenol	MS RT
23	3-Methyl-2-butenol	MS RT
24	Methyl 2-methylbutyrate	MS RT
25	*cis*-2-Pentenal (tent.)	MS
26	Mesityl-oxide	MS RT

27	Hexanal	MS RT
28	Ethyl butyrate	MS RT
29	Butyric acid	MS RT
30	Furfural	MS RT
31	Acetal of 3-penten-2-one (tent.)	MS
32	4-Methylpentanol	MS
33	*trans*-2-hexenal	MS RT
34	Unknown	
35	*cis*-3-Hexenol	MS RT
36	Propyl 2-methylpropanoate	MS RT
37	1,3-Dimethylbenzene	MS RT
38	*trans*-2-Hexenol	MS RT
39	Hexanol	MS RT
40	3-Methylbutyl acetate	MS RT
41	Styrene	MS RT
42	1,2-Dimethylbenzene	MS RT
43	Nonane	MS RT
44	*trans*-2-Heptenal/Benzaldehyde	MS RT
45	Heptanol	MS RT
46	Octen-3-one	MS RT
47	Octen-3-ol	MS RT
48	6-Methyl-5-hepten-2-one	MS RT
49	Decane	MS RT
50	*trans,trans*-2,4-Heptadienal	MS RT
51	Isomer of trimethylbenzene	MS
52	Limonene	MS RT
53	2-Ethylhexanol	MS RT
54	Salicylaldehyde	MS RT

55	Phenylacetaldehyde	MS RT
56	*trans*-2-Octenal	MS RT
57	*cis*-2-Octenol	MS RT
58	Octanol	MS RT
59	*cis*-Linalool-oxide (furan)	MS RT
60	Linalool	MS RT
61	Undecane	MS RT
62	Nonanal	MS RT
63	Unknown	
64	*trans*-2-Nonenal	MS RT
65	Nonanol	MS RT
66	Naphthalene/Terpinen-4-ol	MS RT
67	*p*-Methylacetophenone	MS RT
68	Octanoic acid	MS RT
69	α-Terpineol	MS RT
70	Dodecane	MS RT
71	Decanal	MS RT
72	Benzothiazole	MS RT
73	Quinoline	MS RT
74	Unknown	
75	Unknown	
76	Cinnamaldehyde	MS RT
77	Nonanoic acid	MS RT
78	Isomer of methylnaphthalene	MS
79	Tridecane	MS RT
80	Isomer of methylnaphthalene	MS
81	*trans,trans*-2,4-Decadienal	MS RT
82	Triacetin	MS

83	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid ester (1-hydroxy unbound)	MS RT
84	Decanoic acid	MS RT
85	2,2,4-Trimethyl-1,3-pentanediol2-methylpropanoic acid ester (3-hydroxy unbound)	MS RT
86	Tetradecane	MS RT
87	Butylated hydroxytoluene (B.H.T.)	MS RT
88	Pentadecane	MS RT
89	Unknown	
90	Tributyl phosphate : Plasticiser	MS
91	Dodecanoic acid	MS RT
92	2,2,4-Trimethyl-1,3-pentanediol 2-methylpropanoic acid diester	MS RT
93	Unknown sesquiterpene	MS
94	Guaiol	MS
95	Heptadecane	MS RT
96	Unknown sesquiterpene	MS
97	Tetradecanoic acid	MS RT
98	Unknown alkane	
99	Hexyl decanoate	MS RT
100	Pentadecanoic acid	MS RT
101	2-Methylpropyl tetradecanoate	MS RT
102	Hexadecanoic acid	MS RT
103	Linoleic acid	MS RT
104	Linolenic acid	MS RT

5 The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles

The results shown here are the mean of three analyses for each picking of rhubarb, with extraction of the three samples occurring simultaneously. All samples were collected before 9 A.M. on 27th May, 9th June, 27th June, 11th July and 18th July 1991, and their concentrated extracts numbered 1, 2, 3, 4, 5 respectively (Table IV.5). Samples were taken from the same plants throughout, ensuring that the concentration of volatiles from successively produced stalks was measured.

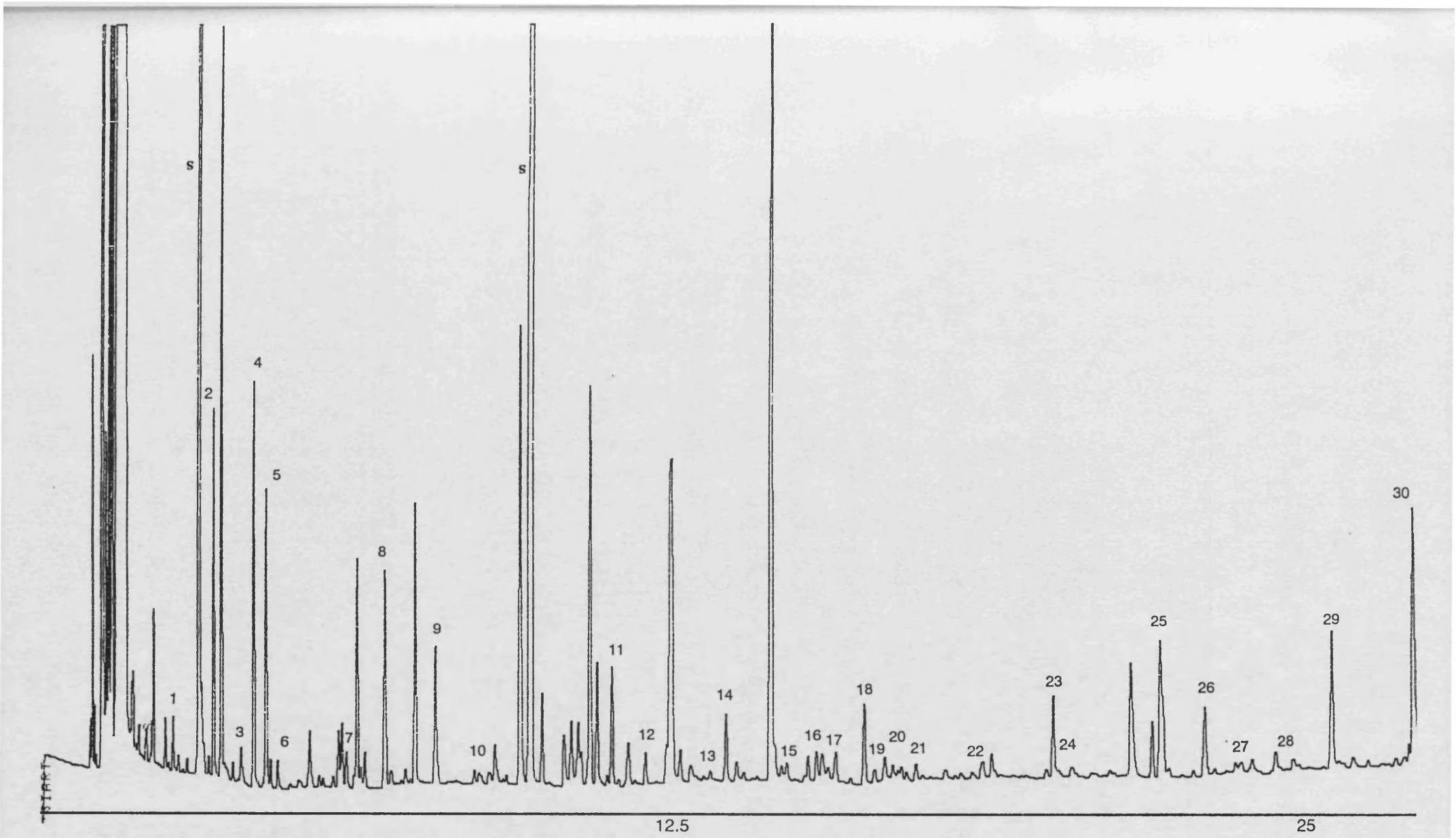
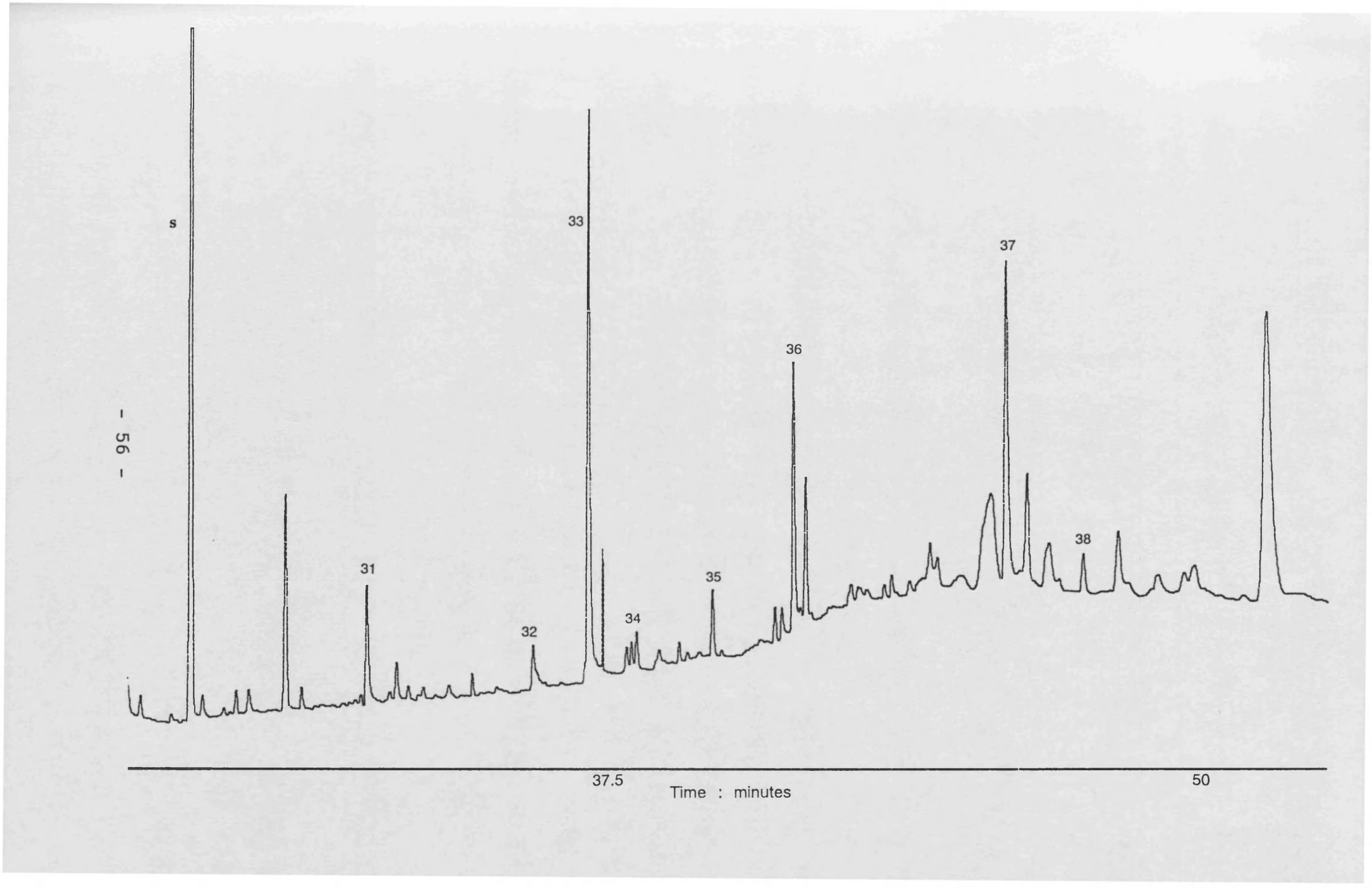


Fig.D.5 Example of a liquid/liquid dichloromethane extract of rhubarb (var. Steins Champagne) on DB-Wax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38
37.5
50
Time : minutes

Table IV.5

Concentration of volatile components in rhubarb stalk (var. Steins Champagne) during the growing season($\mu g\ kg^{-1}$).

VOLATILE COMPONENT	RHUBARB SAMPLE				
	1	2	3	4	5
1 Hexanal	45	30	15	18	32
2 Butanol	92	93	195	80	114
3 Heptanal	589	300	68	trc	26
4 Limonene	184	92	90	66	42
5 2-Methylbutanol	114	93	97	46	53
6 *trans*-2-Hexenal	19	17	16	4	ND
7 Octanal	117	91	69	59	290
8 *trans*-2-Heptenal	186	150	91	48	trc
9 Hexanol	44	51	52	44	47
10 Nonanal	261	207	180	147	199
11 2,4-Heptadienal	65	54	63	20	11
12 Benzaldehyde	28	26	24	12	11
13 Linalool	15	10	3	trc	ND
14 Octanol	410	212	35	67	182

VOLATILE COMPONENT	RHUBARB SAMPLE				
	1	2	3	4	5
15 γ-Butyrolactone	107	60	trc	9	34
16 Phenylacetaldehyde	117	69	30	29	58
17 *trans*-2-Decenal	106	52	10	17	19
18 Salicylaldehyde	17	34	54	47	50
19 Nonanol	19	11	3	2	11
20 2-Methylbutyric acid	74	52	21	46	17
21 α-Terpineol	351	176	11	5	11
22 *trans*-2-Undecenal	36	16	ND	8	9
23 Butoxyethoxyethanol	244	200	164	139	157
24 2,4-Decadienal	62	12	ND	ND	ND
25 Benzyl alcohol	85	69	73	48	62
26 2-Phenylethanol	48	50	46	29	64
27 Benzothiazole	9	12	1	38	52
28 Heptanoic acid	106	33	19	19	trc
29 Phenol	81	49	62	41	74
30 Octanoic acid	116	175	286	200	297
31 Decanoic acid	226	84	105	85	79

VOLATILE COMPONENT	RHUBARB SAMPLE				
	1	2	3	4	5
32 Benzoic acid	172	58	COELU-TION	88	COELU-TION
33 Dodecanoic acid	1034	1099	758	1418	1813
34 Vanillin	166	75	COELU-TION	37	93
35 Acetovanillone	72	45	136	55	51
36 Tetradecanoic acid	524	783	700	573	1331
37 Hexadecanoic acid	1156	1558	569	804	684
38 Frambinone	40	194	60	59	134
Total Peak Area as % of Sample 1	100%	81%	69%	51%	81%

key

ND : Not Detected

trc : trace

coelution : coelution of more than one component

6 The volatiles derived from the enzymatic hydrolysis of linoleic/linolenic acids in rhubarb

In order to examine the changes in composition of volatiles during homogenate standing, rhubarb stalks were harvested on three successive days and analysed as shown in Chapter 2. The figures below are the means of these three analyses.

Table IV.6

Table showing the level of C_6 volatiles ($\mu g\ kg^{-1}$) against storage time for homogenised rhubarb stalk

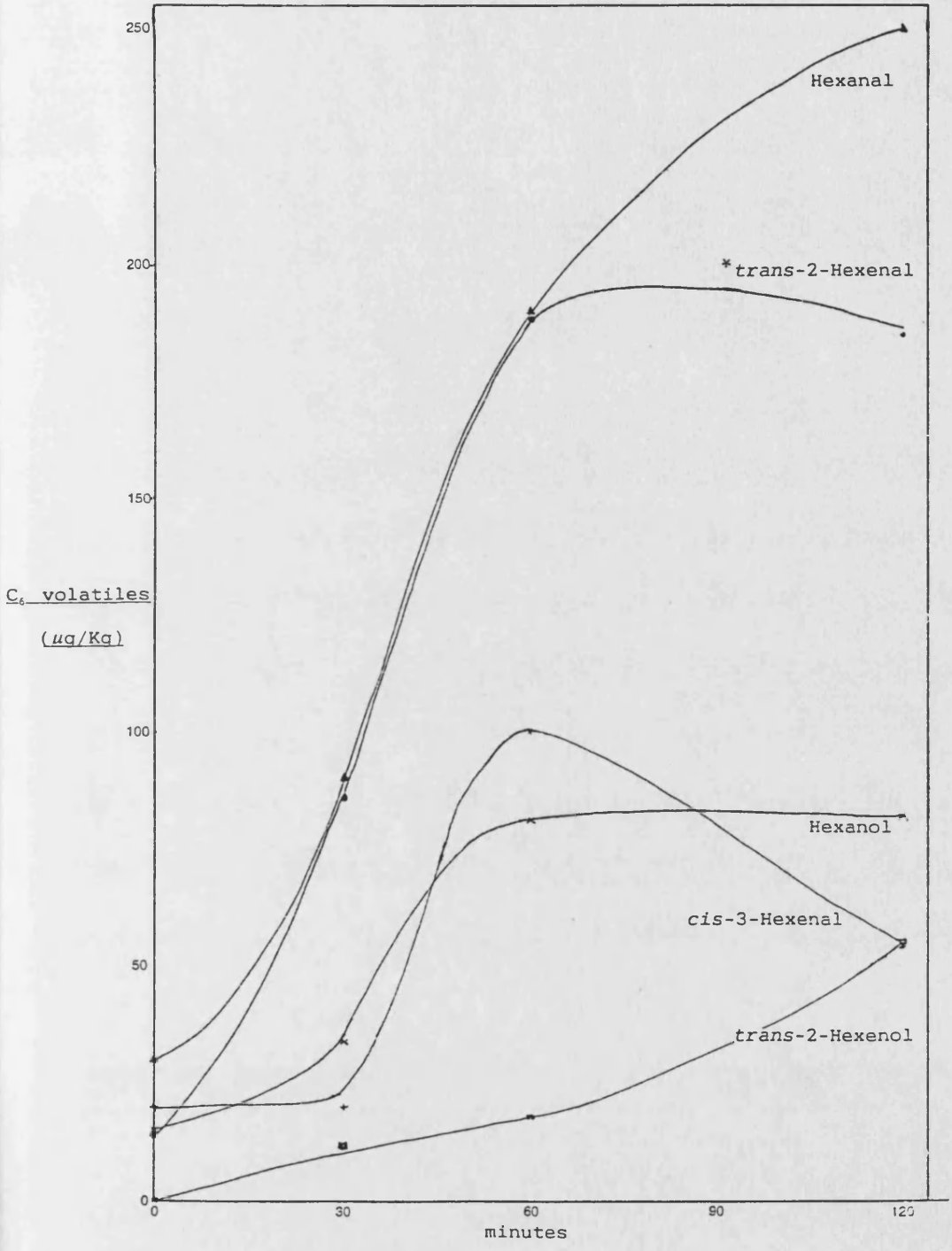
Identified C_6 Volatile	Time before Enzyme Inhibition (mins)			
	0	30	60	120
Hexanal	30	90	190	250
cis-3-Hexenal	20	20	100	54
trans-2-Hexenal	140	860	1920	1850
Hexanol	16	34	81	82
cis-3-Hexenol	trc	trc	ND	ND
trans-2-Hexenol	ND	12	18	55

key

ND : Not Detected

trc : trace

Fig.D.6 Graph showing $\mu g kg^{-1}$ C_6 volatiles in rhubarb stalk produced enzymatically during homogenate standing.



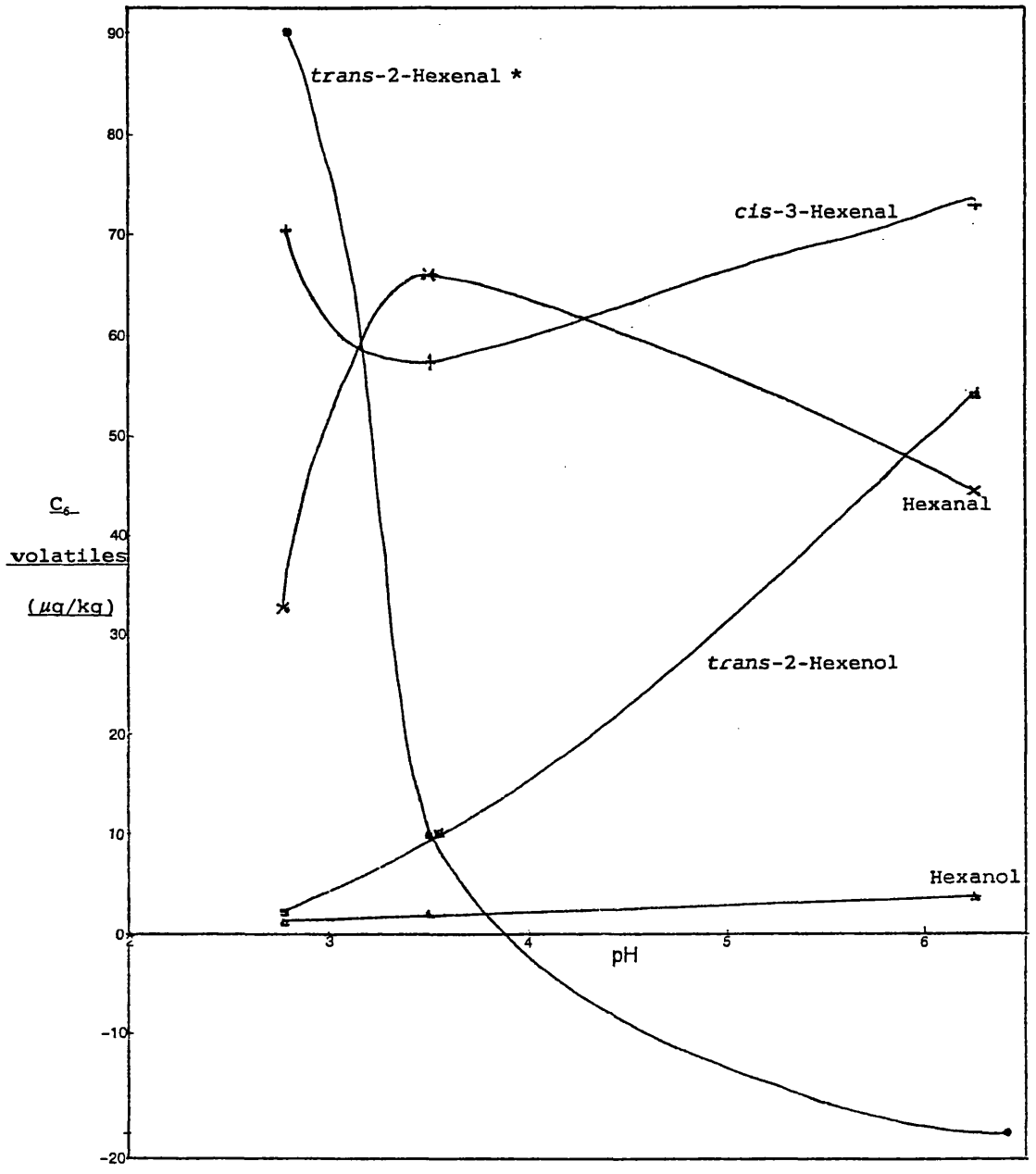
Key * : *trans*-2-Hexenal conc is 10x that shown

Table IV.7

Table showing the level of C_6 volatiles ($\mu g\ kg^{-1}$) against pH value for homogenised rhubarb.

C_6 Volatile	CONTROL time : 0	pH VALUE 2.8	3.5	6.4
Hexanal	25.7	58.3	91.4	69.7
cis-3-Hexenal	42.8	112.9	100.2	115.4
trans-2-Hexenal	360.0	1272.1	460.0	157.3
Hexanol	1.3	1.6	3.2	4.7
trans-2-Hexenol	3.8	5.8	12.6	58.0

Fig.D.7 Graph showing μgkg^{-1} C_6 volatiles rhubarb stalk produced over 90mins at various pH values minus control.



Key * : *trans*-2-Hexenal conc is 10x that shown

CHAPTER 5

ANALYTICAL RESULTS

RHUBARB GLYCOSIDES

Results were obtained for the following analyses:-

1) The enzymatically released aglycones in rhubarb stalk

Chromatogram (DB5 column) : Fig.E.1

Compounds identified : Table V.1

Chromatogram (DB WAX column) : Fig.E.2

Compounds identified : Table V.2

2) The enzymatically released aglycones in rhubarb leaf

Chromatogram (DB5 column) : Fig.E.3

Compounds identified : Table V.3

Chromatogram (DB WAX column) : Fig.E.4

Compounds identified : Table V.4

3) The enzymatically released aglycones in rhubarb root

Chromatogram (DB5 column) : Fig.E.5

Compounds identified : Table V.5

Chromatogram (DB WAX column) : Fig.E.6

Compounds identified : Table V.6

In all tables peak identifications are labelled MS and RT. MS indicates that the component has been identified by mass spectral data. RT indicates that the component has been shown to coelute with an authentic sample of the chemical.

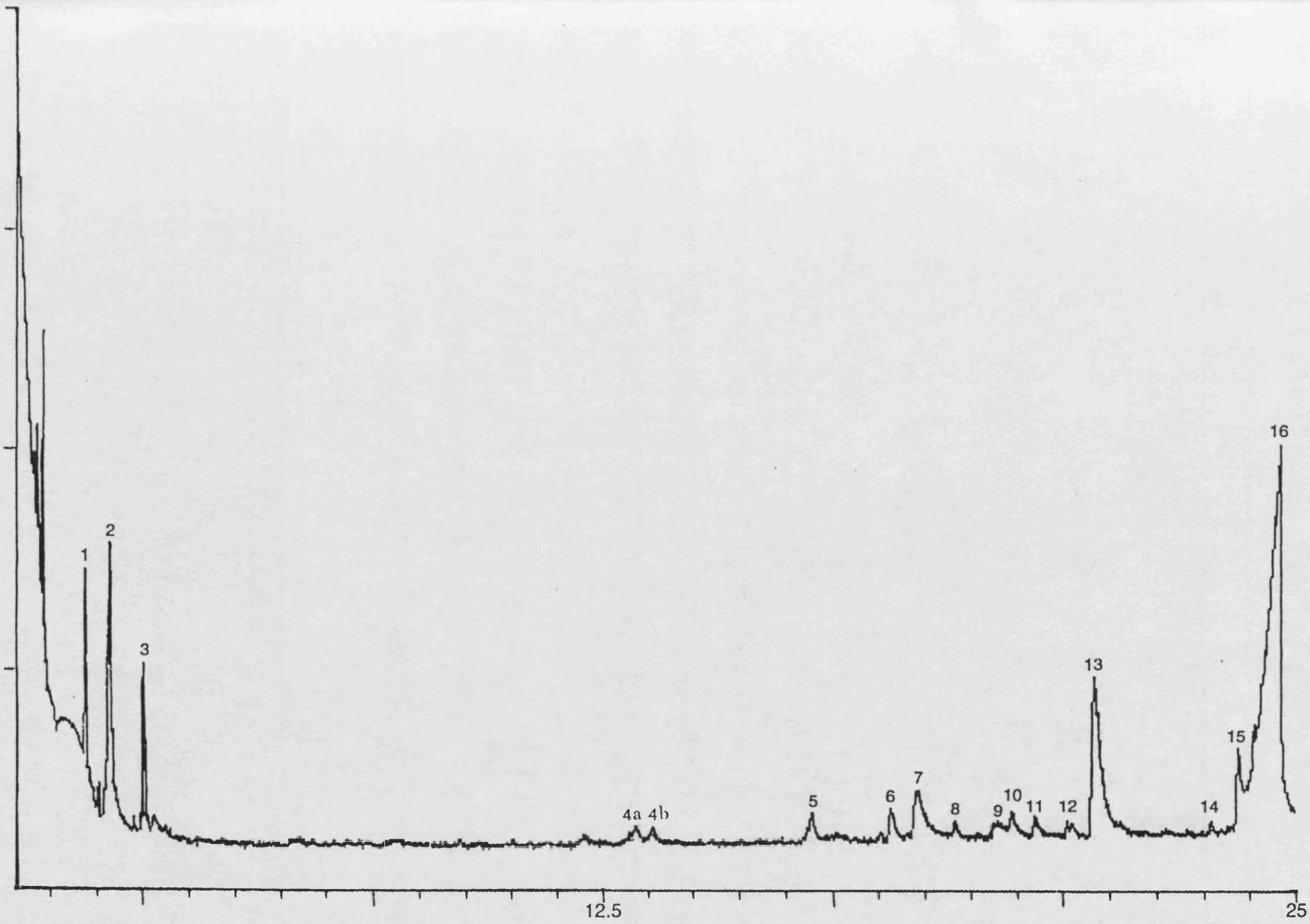
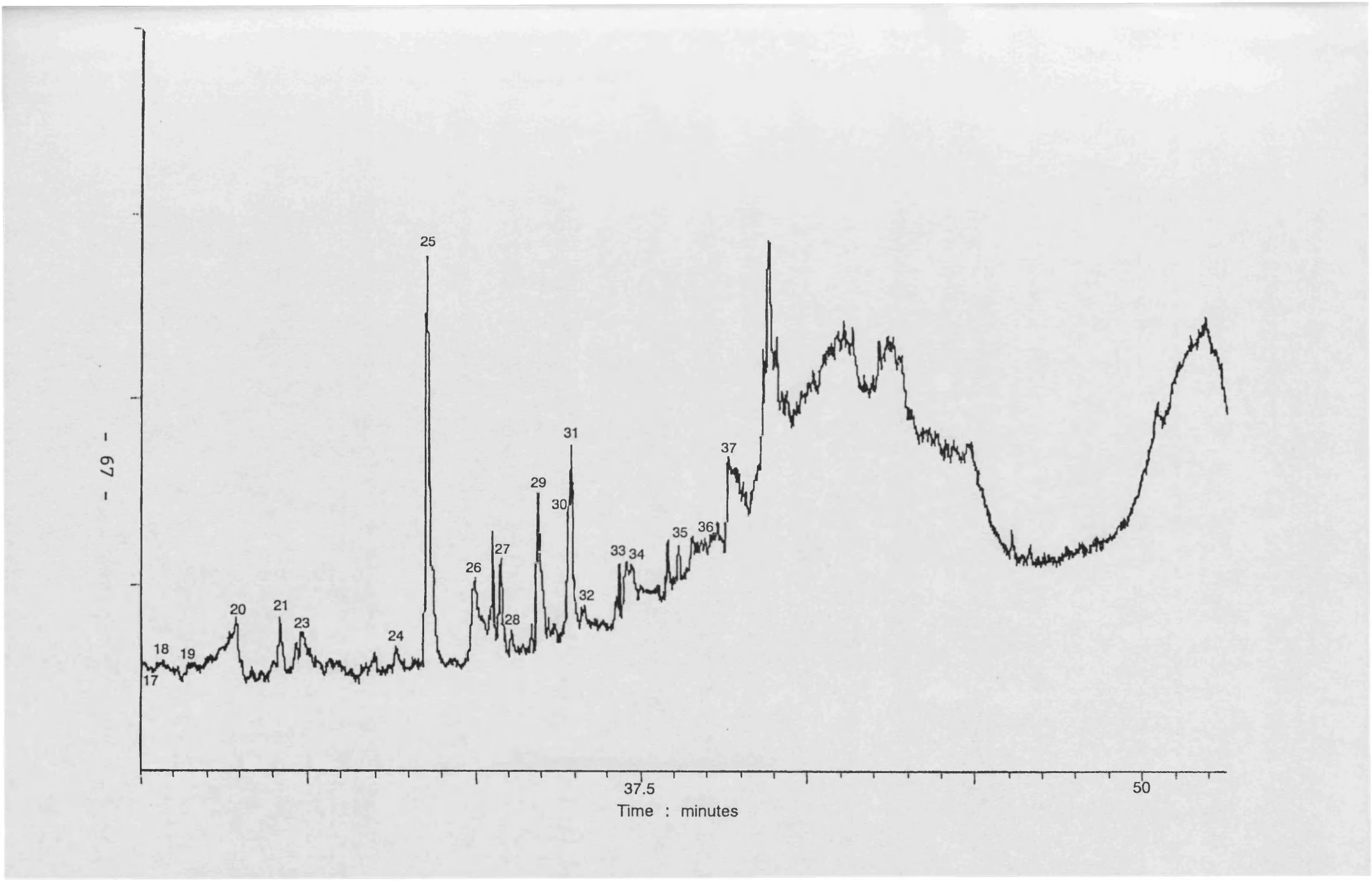


Fig.E.1 GC trace of the rhubarb stalk aglycones analysed on a DB-5 column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36
37
37.5
50
Time : minutes

Table V.1 Peak identification: rhubarb stalk aglycones on DB-5 column

1	2-Methylbutanol	MS RT
2	Pyridine	MS RT
3	Toluene	MS RT
4a	5-Methylhexanol	MS RT
4b	Benzaldehyde	MS RT
5	Octanal	MS RT
6	Limonene	MS RT
7	Benzyl alcohol	MS RT
8	-	
9	*o*-Cresol	MS RT
10	Octanol	MS RT
11	*cis*-Linalool-oxide (furan)	MS RT
12	-	

13	2-Phenylethanol	MS RT
14	-	
15	2-(2-Butoxyethoxy)ethanol	MS RT
16	Octanoic acid/Naphthalene	MS RT
17	-	
18	4-Vinylphenol	MS RT
19	Anisaldehyde	MS RT
20	4-Vinylguaiacol	MS RT
21	Eugenol	MS RT
22	-	
23	Isomer of dimethylnaphthalene	MS
24	Frambinone	MS RT
25	-	
26	Dodecanoic acid / 3,4,5-Trimethoxybenzaldehyde	MS RT

27	4-Allyl-2,6-dimethoxyphenol	MS
28	3-Hydroxy-β-damascone (tent.)	MS
29	Zingerone	MS RT
30	4-Oxo-β-ionol	MS RT
31	3-Hydroxy-5,6-epoxy-β-ionone	MS
32	-	
33	-	
34	Tetradecanoic acid	MS RT
35	Methyl vanillate	MS RT
36	-	
37	-	

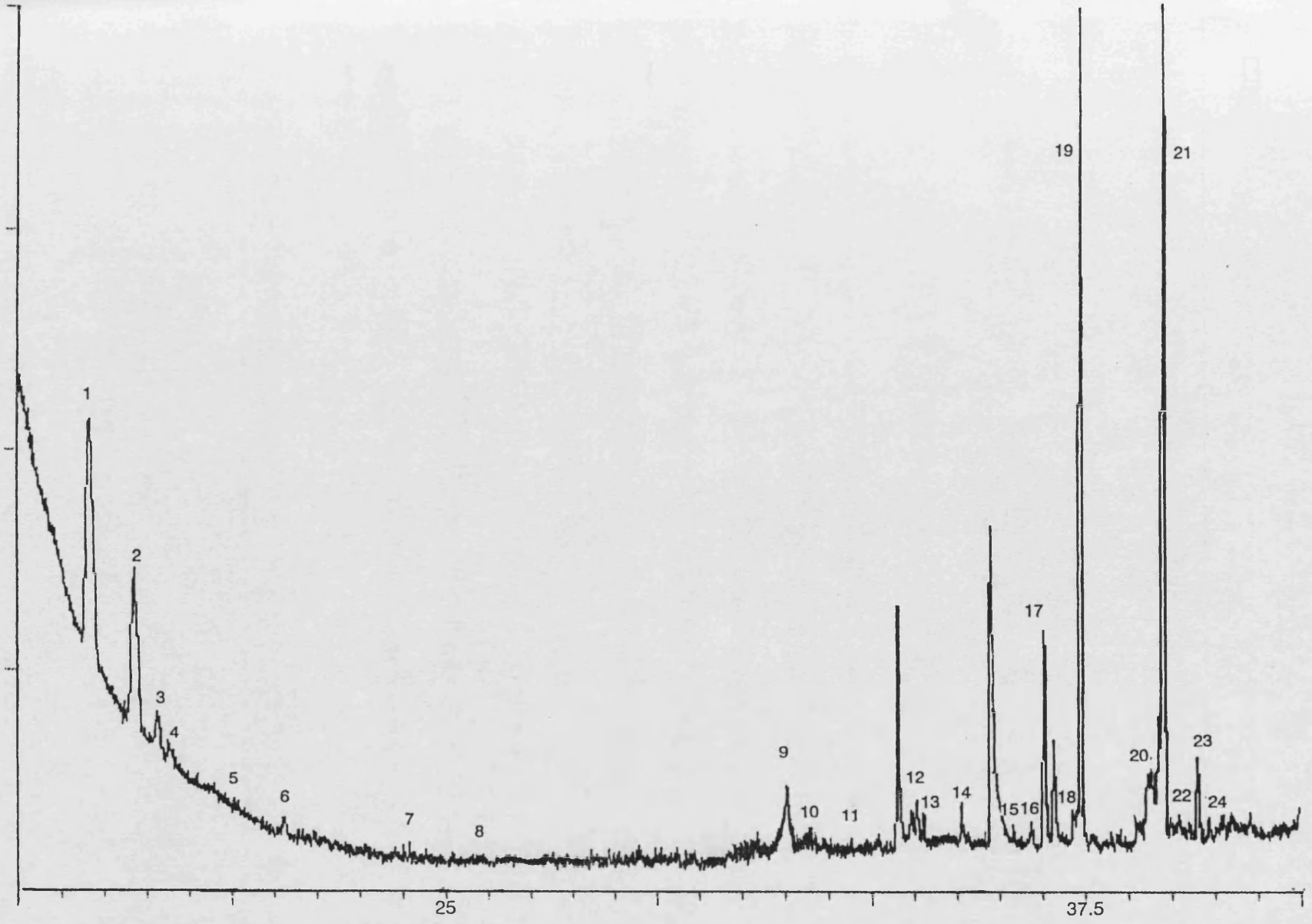
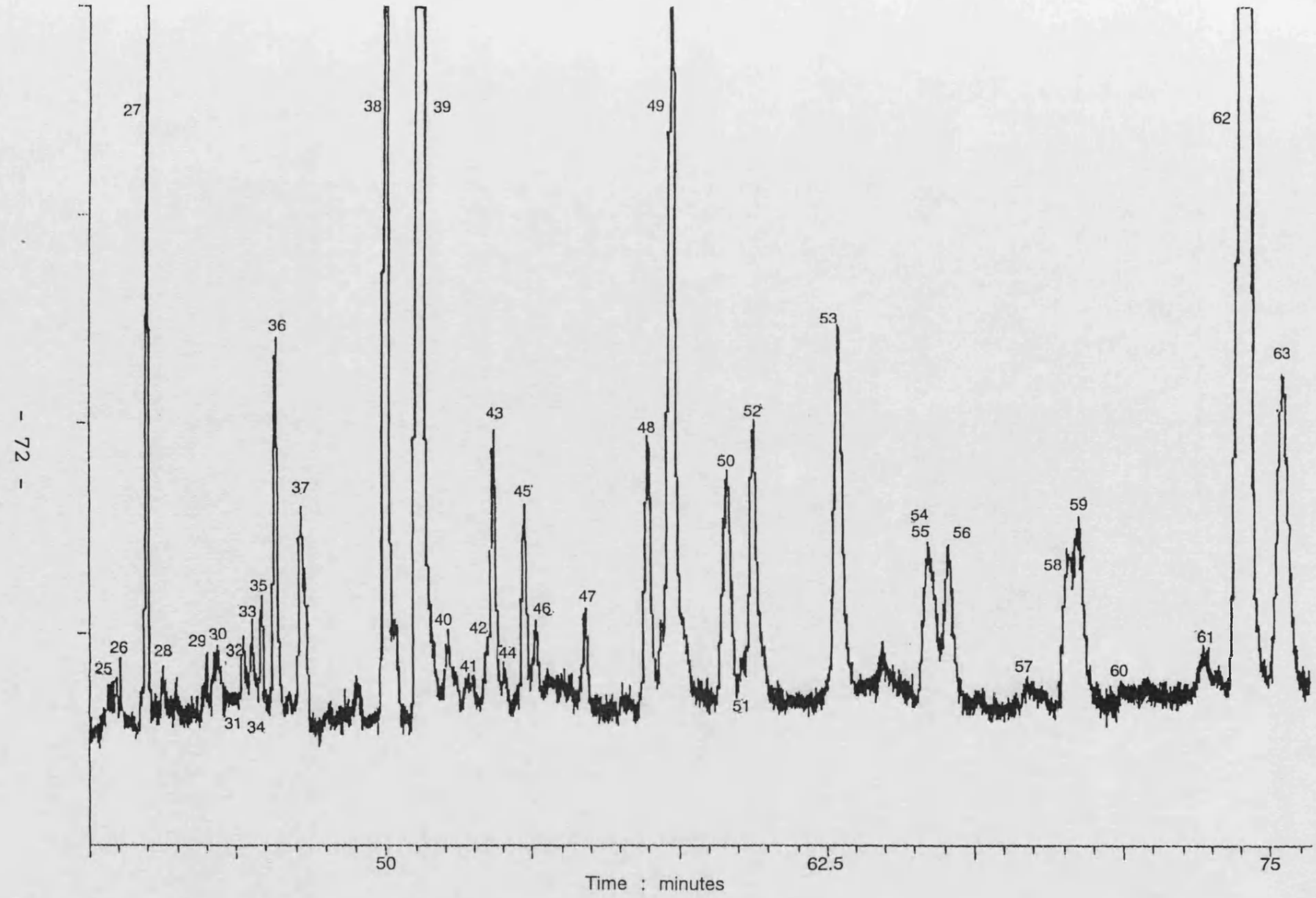


Fig.E.2 GC trace of the rhubarb stalk aglycones analysed on a DB-Wax column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61
62
63
50
62.5
75
Time : minutes

Table V.2 Peak identification: rhubarb stalk aglycones on DB-WAX column

1	1,4-Cineol	MS
2	Limonene	MS RT
3	1,8-Cineol	MS RT
4	2-Methylbutanol	MS RT
5	3-Methylpentanol	MS RT
6	*p*-Cymene/Octanol	MS RT
7	Hexanol	MS RT
8	2-Butoxyethanol	MS
9	Acetic acid	MS RT
10	*trans*-Linalool-oxide (pyran)	MS RT
11	Octanol	MS RT
12	Naphthalene	MS RT
13	*cis*-Linalool-oxide (pyran)	MS RT

14	2-(2-Butoxyethoxy)ethanol	MS RT
15	Hexanoic acid/Unknown	MS RT
16	*cis*-Carveol	MS RT
17	Benzyl alcohol	MS RT
18	Butylated hydroxytoluene (B.H.T)	MS RT
19	2-Phenylethanol	MS RT
20	*o*-Cresol	MS RT
21	Phenol	MS RT
22	Isomer of dimethylnapthalene	MS
23	Anisaldehyde	MS RT
24	*p*-Cresol	MS RT
25	-	
26	Eugenol	MS RT
27	4-Vinylguaiacol	MS RT

28	-	
29	Isomer of dimethoxyphenol	MS
30	2,6-Dimethoxyphenol	MS RT
31	Isomer of dimethoxyphenol	MS
32	Cinnamyl alcohol	MS RT
33	-	
34	-	
35	-	
36	Methyl 4-hydroxybenzoate (tent.)	MS
37	4-Propenylphenol	MS RT
38	4-Vinylphenol	MS RT
39	-	
40	-	
41	-	

42	Indole	MS RT
43	Unknown norisoprenoid	MS
44	Benzoic acid/Unknown *d*-lactone	MS RT
45	-	
46	Phenylacetic acid	MS RT
47	-	
48	3,4,5-Trimethoxybenzaldehyde	MS
49	4-Allyl-2,6-dimethoxyphenol	MS RT
50	Vanillin	MS RT
51	Unknown sesquiterpene 204 MW?	MS
52	2,6-Dimethoxy-4-vinylphenol	MS
53	Methyl vanillate	MS RT
54	3-Oxo-α-ionol	MS RT
55	Acetovanillone	MS RT

56	4-Oxo-β-ionol	MS RT
57	-	
58	4-Oxo-7,8-dihydro-β-ionol	MS
59	3-Hydroxy-β-ionone	MS
60	-	
61	Unknown.195 m/z base peak	MS
62	3-Hydroxy-5,6-epoxy-β-ionone	MS
63	Zingerone	MS RT
64	Frambinone	MS RT
65	-	
66	-	

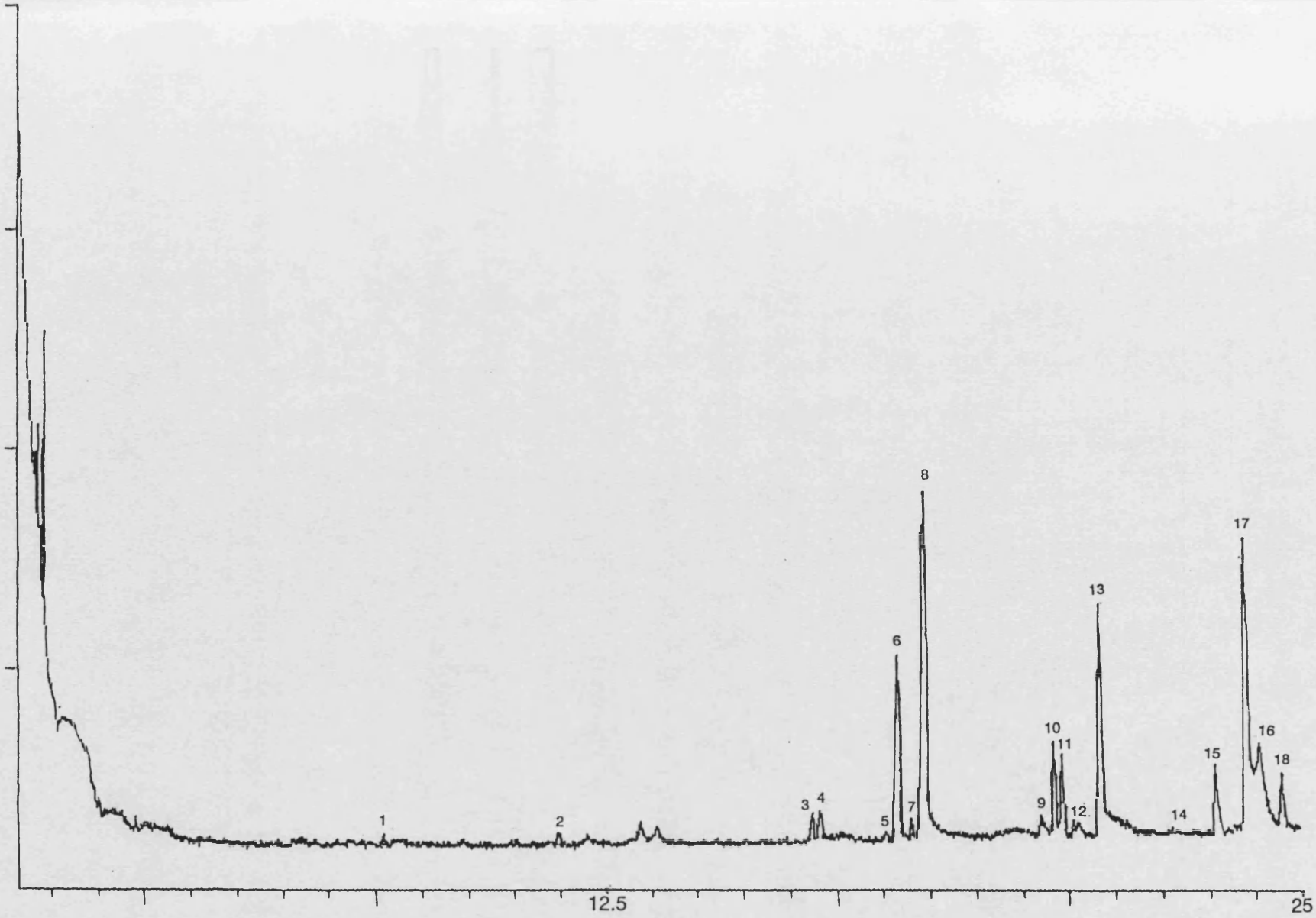
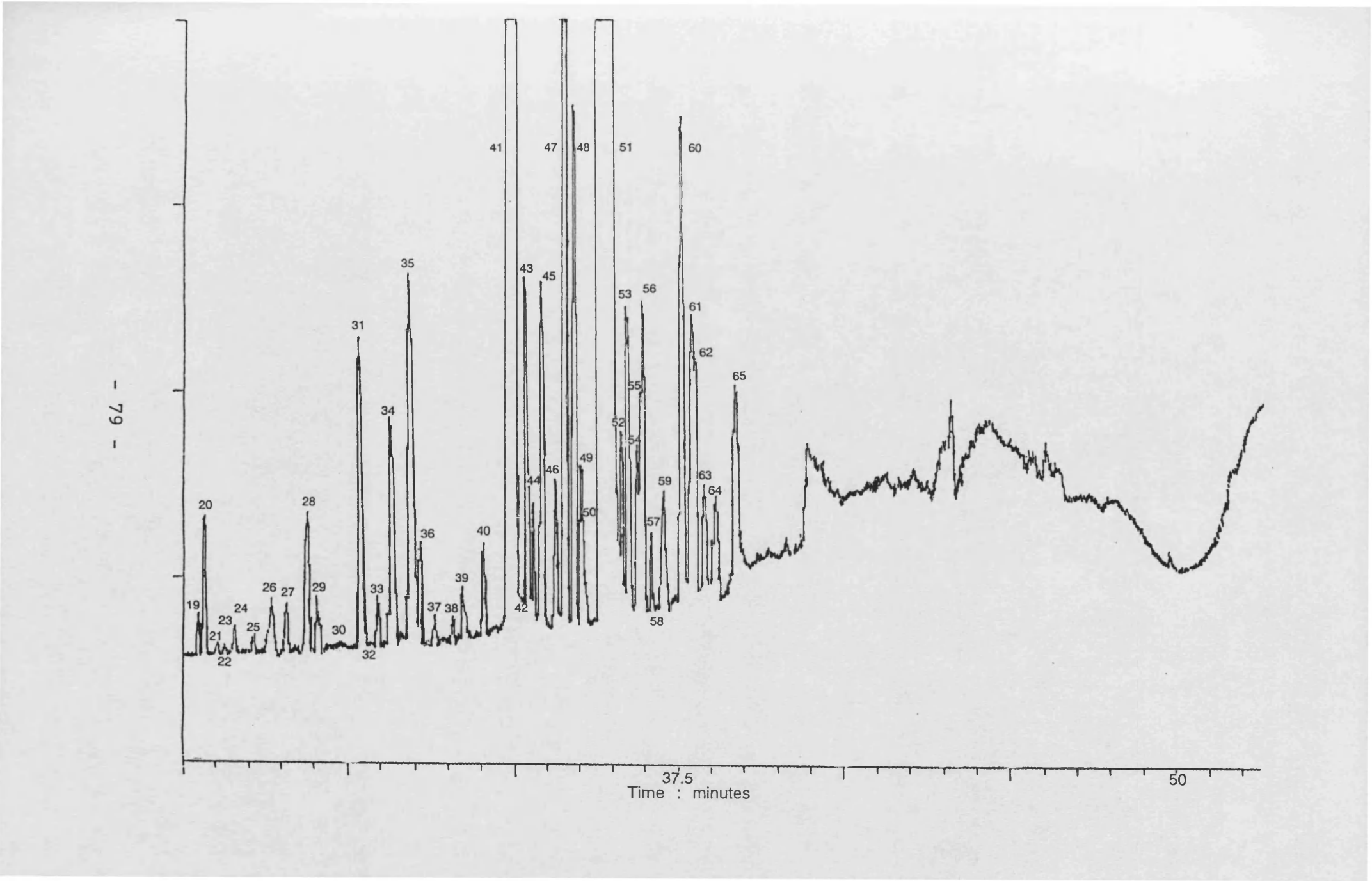


Fig.E.3 GC trace of the rhubarb leaf aglycones analysed on a DB-5 column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59
60
61
62
63
64
65
37.5
50
Time : minutes

Table V.3 Peak identification: rhubarb leaf aglycones on DB-5 column

1	1,3-Dimethylbenzene	MS
2	-	
3	Phenol	MS RT
4	Butyric acid/Unknown	MS RT
5	Hexanoic acid	MS RT
6	Limonene	MS RT
7	*trans*-2-Hexenoic acid	MS RT
8	Benzyl alcohol	MS RT
9	*cis*-Linalool-oxide	MS RT
10	Octanol	MS RT
11	-	
12	-	
13	2-Phenylethanol	MS RT

14	Naphthalene	MS RT
15	Benzoic acid	MS RT
16	Octanoic acid	MS RT
17	2-(2-Butoxyethoxy)ethanol	MS RT
18	-	
19	-	
20	4-Vinylphenol	MS RT
21	-	
22	-	
23	Anisaldehyde	MS RT
24	-	
25	Indole	MS RT
26	4-Vinylguaiacol	MS RT
27	-	

28	2,6-Dimethoxyphenol	MS RT
29	1,2-Dihydro-1,1,6-trimethylnaphthalene	MS
30	-	
31	Vanillin	MS RT
32	*cis*-Isoeugenol	MS RT
33	Isomer of dimethylnaphthalene	MS
34	Isomer of dehydroionone (tent.)	MS
35	*trans*-Isoeugenol	MS RT
36	1-(4-Hydroxy-3-methoxyphenyl)ethanol	MS
37	Acetovanillone	MS RT
38	-	
39	Guaiacylacetone	MS
40	Frambinone	MS RT
41	Dodecanoic acid	MS RT

42	-	
43	-	
44	-	
45	4-Allyl-2,6-dimethoxyphenol	MS RT
46	3-Hydroxy-β-damascone(tent.)/unknown	MS
47	-	
48	3-Oxo-α-ionol	MS RT
49	3,4-Didehydro-β-ionol	MS
50	Zingerone/Syringaldehyde	MS RT
51	3-Hydroxy-5,6-epoxy-β-ionone	MS
52	-	
53	-	
54	4-Oxo-7,8-dihydro-β-ionol	MS
55	-	

56	-	
57	-	
58	-	
59	Tetradecanoic acid	MS RT
60	-	
61	-	
62	Dehydrovomifoliol	MS
63	-	
64	-	
65	-	

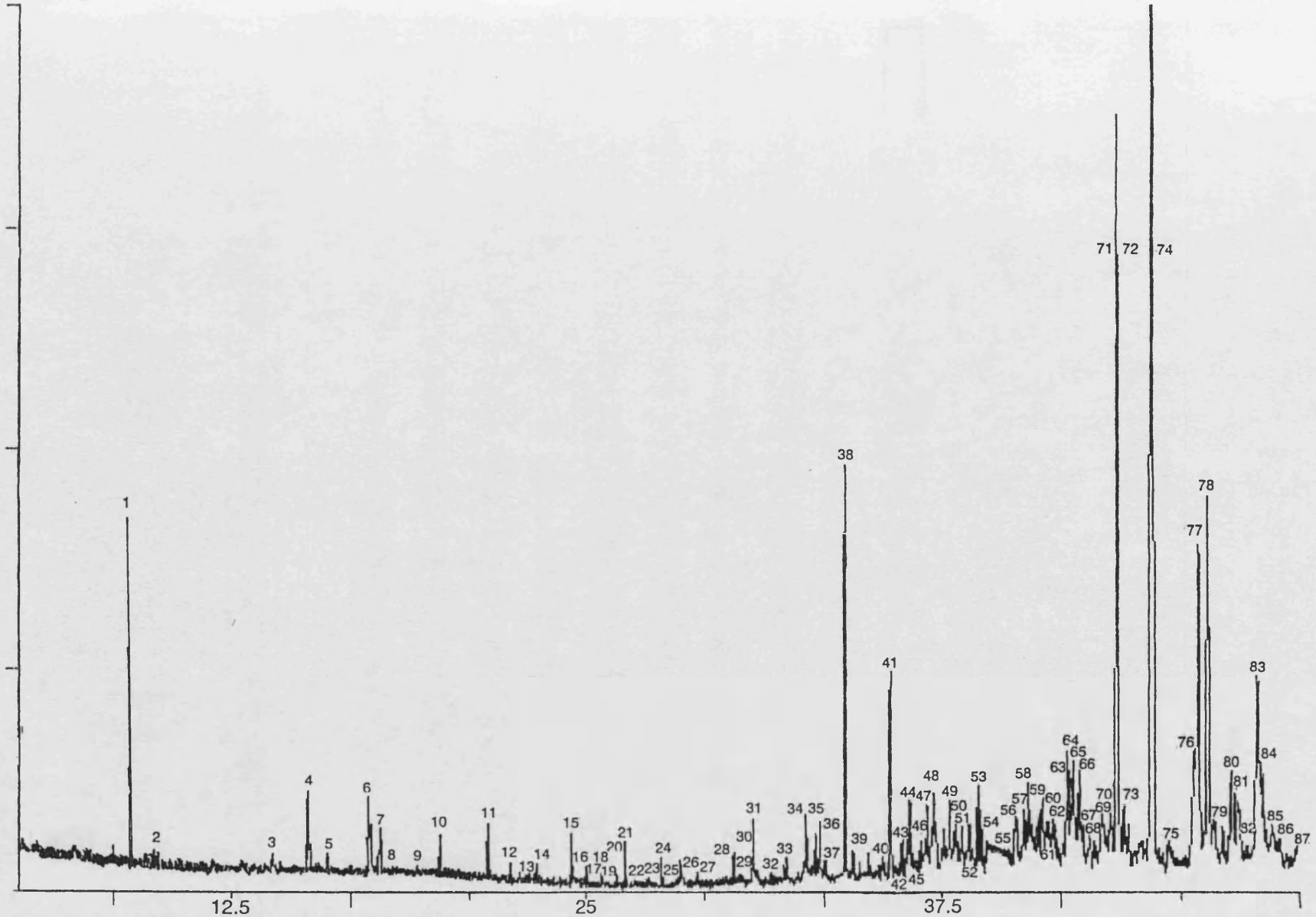
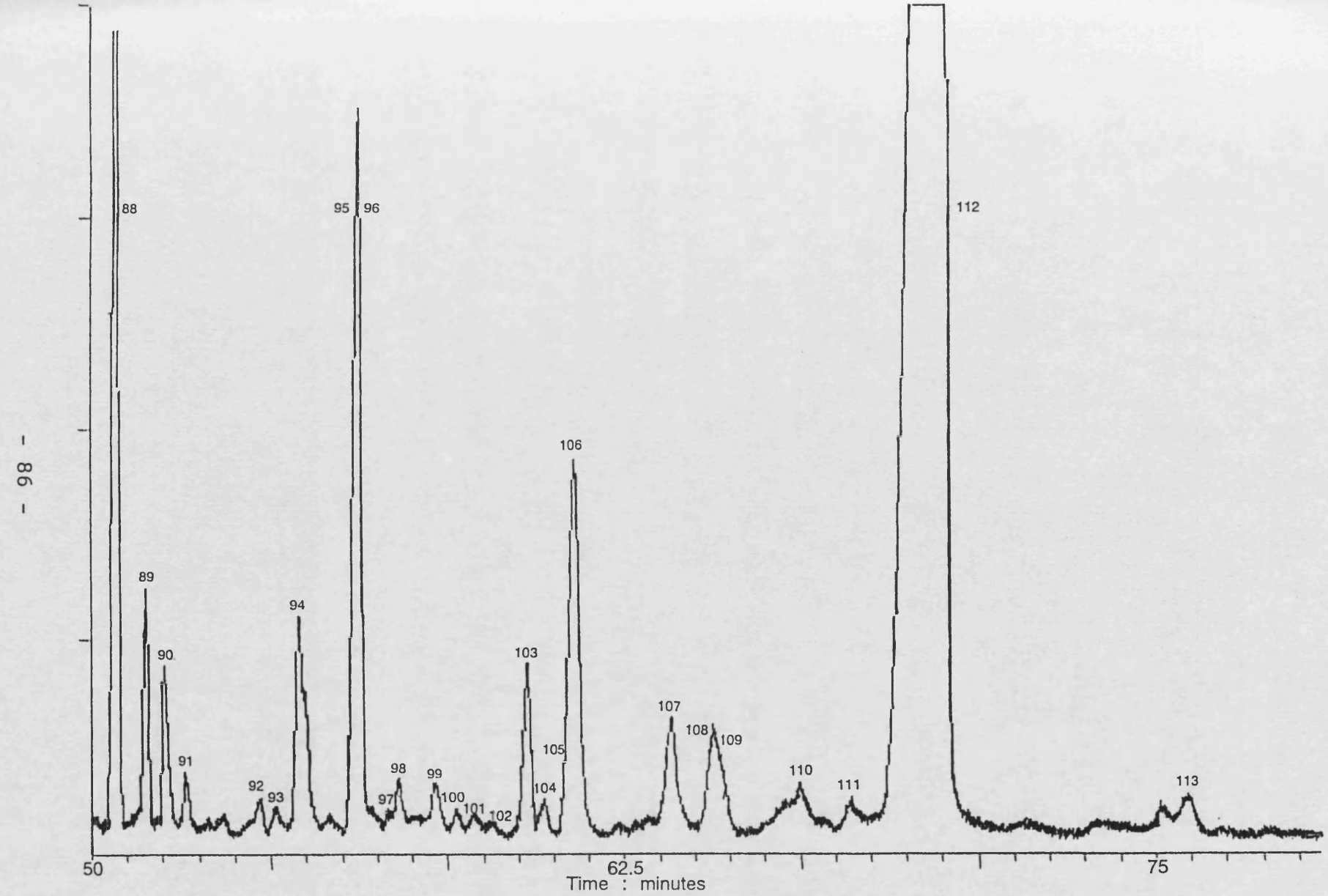


Fig.E.4 GC trace of the rhubarb leaf aglycones analysed on a DB-Wax column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108
109
110
111
112
113
50
62.5
75
Time : minutes

Table V.4 Peak identification: rhubarb leaf aglycones on DB-WAX column

1	3-Penten-2-one	MS RT
2	2-Methyl-3-buten-2-ol	MS RT
3	-	
4	3-Penten-2-ol	MS RT
5	-	
6	1,3-Dimethylbenzene	MS
7	Butanol	MS RT
8	-	
9	-	
10	Limonene	MS RT
11	3-Methylpentanol	MS RT
12	*p*-Cymene/Octanal	MS RT
13	-	

14	Hexanol	MS. RT
15	*cis*-3-Hexenol	MS RT
16	*trans*-2-Hexenol	MS RT
17	Acetic acid	MS RT
18	Heptanol	MS RT
19	-	
20	1-(2-Methoxy-1-methylethoxy)-2-propanol(tent.)	MS
21	*trans*-Linalool-oxide (furan)	MS RT
22	2-Ethylhexanol	MS RT
23	Benzaldehyde	MS RT
24	1-(2-Methoxypropoxy)-2-propanol(tent.)	MS
25	Unknown norisoprenoid 192 MW?	MS
26	Unknown norisoprenoid 192 MW?	MS
27	Octanol	MS RT

28	-	
29	-	
30	-	
31	2,2,6,8-Tetramethyl-7,11-dioxatricyclo-[6.2.1.0]-undec-4-ene	MS
32	Butyric acid	MS RT
33	-	
34	2-Methylbutyric acid	MS RT
35	-	
36	1,2-Dihydro-1,1,6-trimethylnaphthalene	MS
37	*cis*-Linalool-oxide (pyran)	MS RT
38	2-(2-Butoxyethoxy)ethanol	MS RT
39	Hexanoic acid	MS RT
40	Guaiacol	MS RT
41	Benzyl alcohol	MS RT

42	-	
43	-	
44	2-Phenylethanol	MS RT
45	-	
46	Unknown norisoprenoid (dehydroionone isomer?)	MS
47	*cis*-3-Hexenoic acid	MS RT
48	*trans*-2-Hexenoic acid	MS RT
49	-	
50	Phenol	MS RT
51	Isomer of dimethylnaphthalene	MS
52	Anisaldehyde	MS RT
53	-	
54	Octanoic acid	MS RT
55	*p*-Cresol	MS RT

56	Isomer of trimethylnaphthalene	MS
57	-	
58	-	
59	-	
60	Unknown norisoprenoid MW 196?	MS
61	Eugenol	MS RT
62	-	
63	-	
64	-	
65	4-Vinylguaiacol	MS RT
66	-	
67	Unknown norisoprenoid MW 192?	MS
68	Isomer(s) of trimethylphenyl-2-butanone	MS
69	-	

70	2,6-Dimethoxyphenol	MS RT
71	-	
72	-	
73	Unknown 139 MW?	MS
74	Isomer of dimethyloctadienediol	MS
75	Isomer of dimethyloctadienediol	MS
76	-	
77	4-Propenylphenol	MS RT
78	-	
79	-	
80	4-Vinylphenol	MS RT
81	-	
82	-	
83	Benzoic acid	MS RT

84	-	
85	-	
86	-	
87	-	
89	-	
90	Dodecanoic acid	MS RT
91	Phenylacetic acid	MS RT
92	-	
93	3,4,5-Trimethoxybenzaldehyde	MS
94	4-Allyl-2,6-dimethoxyphenol	MS RT
95	Vanillin	MS RT
96	3,4-Didehydro-β-ionol	MS
97	2,6-Dimethoxy-4-vinylphenol	MS
98	Unknown norisoprenoid MW 210?	MS

99	Unknown norisoprenoid MW 208?	MS
100	-	
101	Isomer of dihydroxyionone MW 208?	MS
102	-	
103	Unknown norisoprenoid MW 206?	MS
104	-	
105	Acetovanillone	MS RT
106	3-Oxo-α-ionol	MS RT
107	3-Hydroxy-7,8-dihydro-β-ionol	MS
108	4-Oxo-7,8-dihydro-β-ionol	MS
109	3-Hydroxy-β-ionone	MS
110	-	
111	-	
112	3-Hydroxy-5,6-epoxy-β-ionone	MS

113 Zingerone/Unknown MS RT

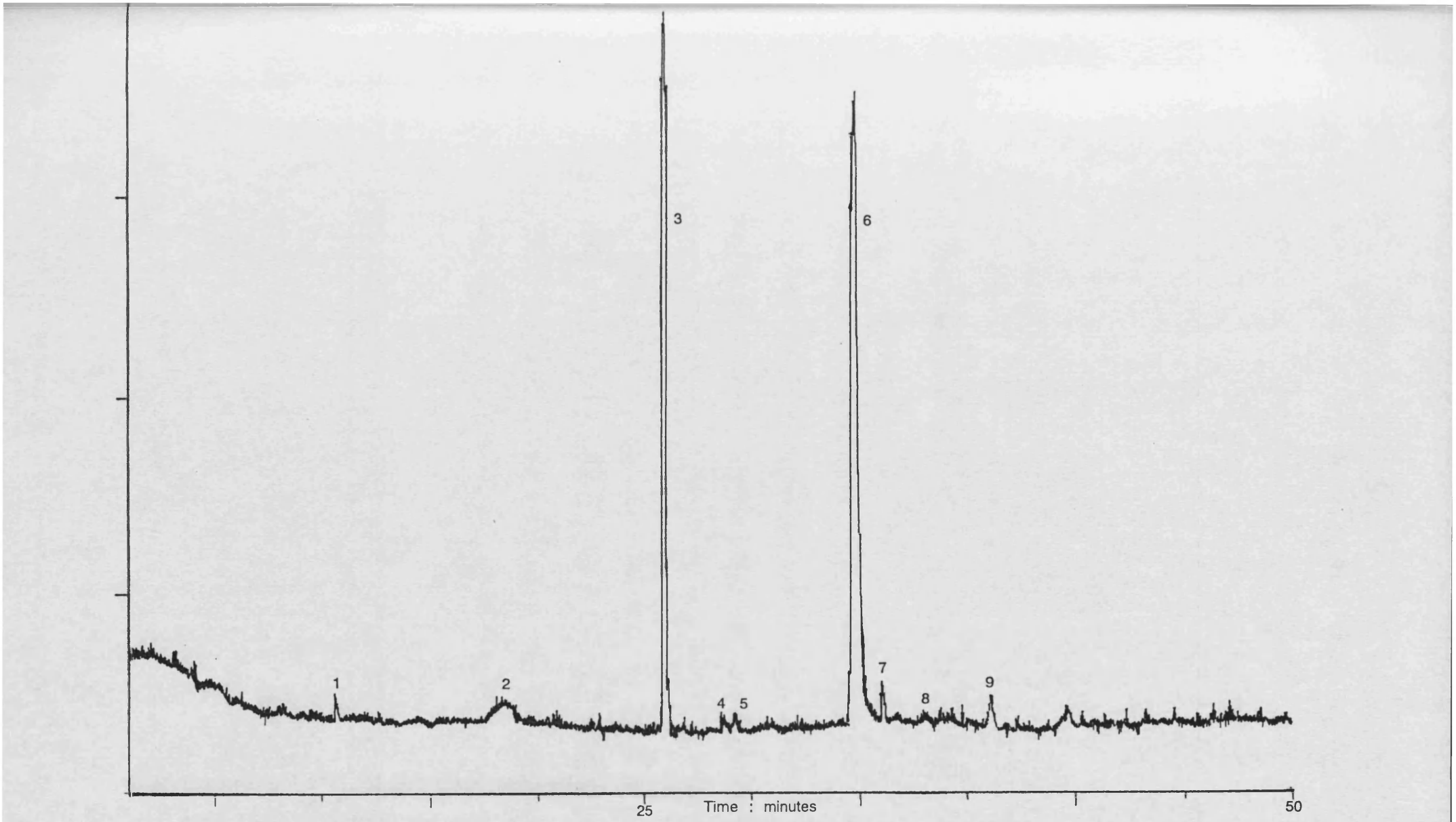


Fig.E.5 GC trace of the rhubarb root aglycones analysed on a DB-5 column

Table V.5 Peak identification: rhubarb root aglycones on DB-5 column

1	Benzaldehyde	MS RT
2	-	
3	Anisaldehyde	MS RT
4	Vanillin	MS RT
5	-	
6	Dodecanoic acid	MS RT
7	4-Allyl-2,6-dimethoxyphenol	MS RT
8	Methyl vanillate	MS RT
9	-	

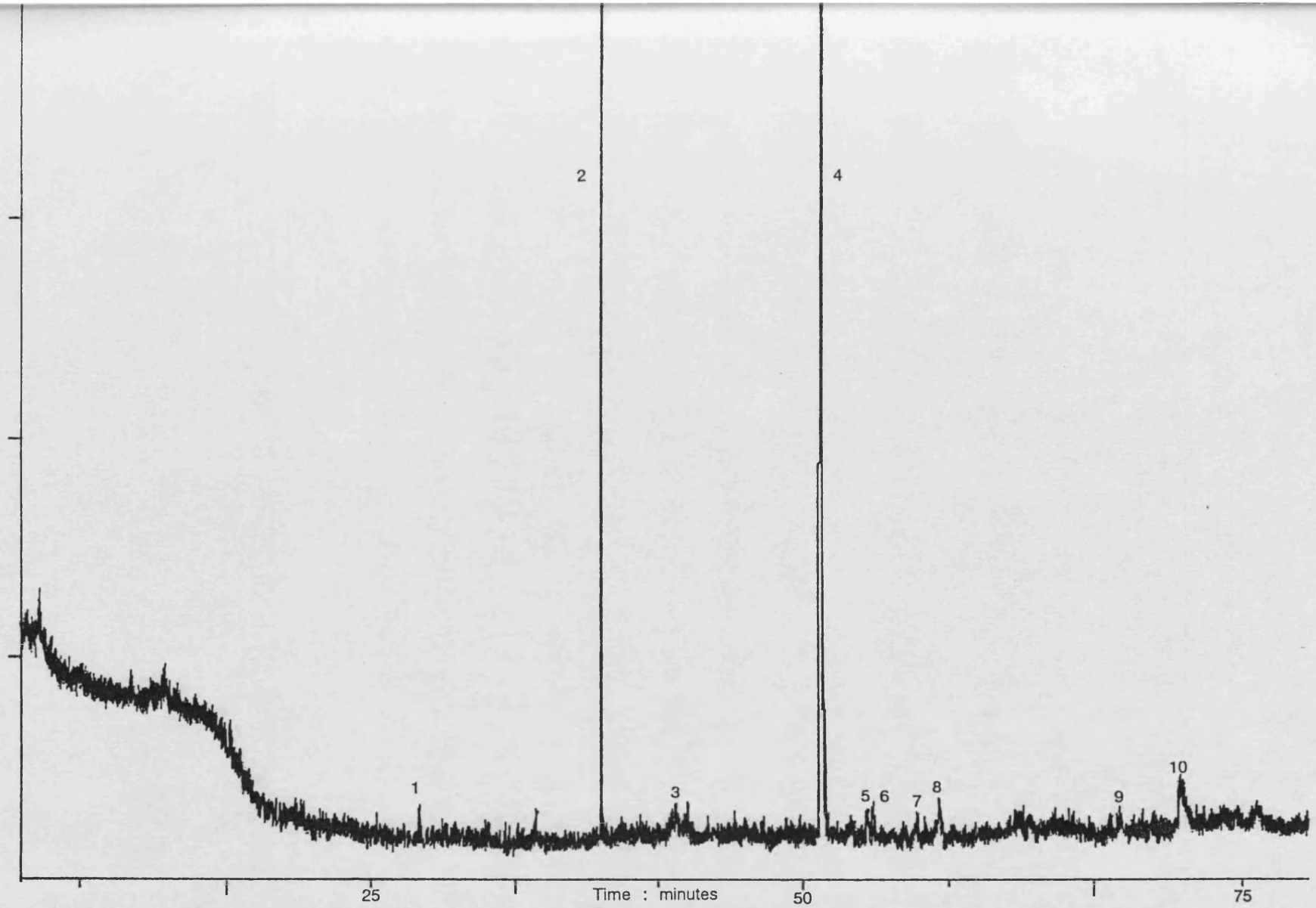


Fig.E.6 GC trace of the rhubarb root aglycones analysed on a DB-WAX column

Table V.6 Peak identification: rhubarb root aglycones on DB-WAX column

1	Benzaldehyde	MS RT
2	Anisaldehyde	MS RT
3	-	
4	Dodecanoic acid	MS RT
5	4-Allyl-2,6-dimethoxyphenol	MS RT
6	-	
7	Vanillin	MS RT
8	Methyl vanillate	MS RT
9	-	
10	-	

CHAPTER 6

DISCUSSION OF RESULTS: RHUBARB VOLATILES

The following subjects are covered in this discussion:-

1) **Comparison of the cold dichloromethane, distilled and preheated rhubarb extracts.**

   A) Volatiles present in intact rhubarb.

   B) The rhubarb volatiles which develop during homogenisation and heating.

2) **The dichloromethane extraction of canned rhubarb.**

3) **The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles.**

4) **The volatiles derived from the enzymatic hydrolysis of linoleic/linolenic acids in rhubarb.**

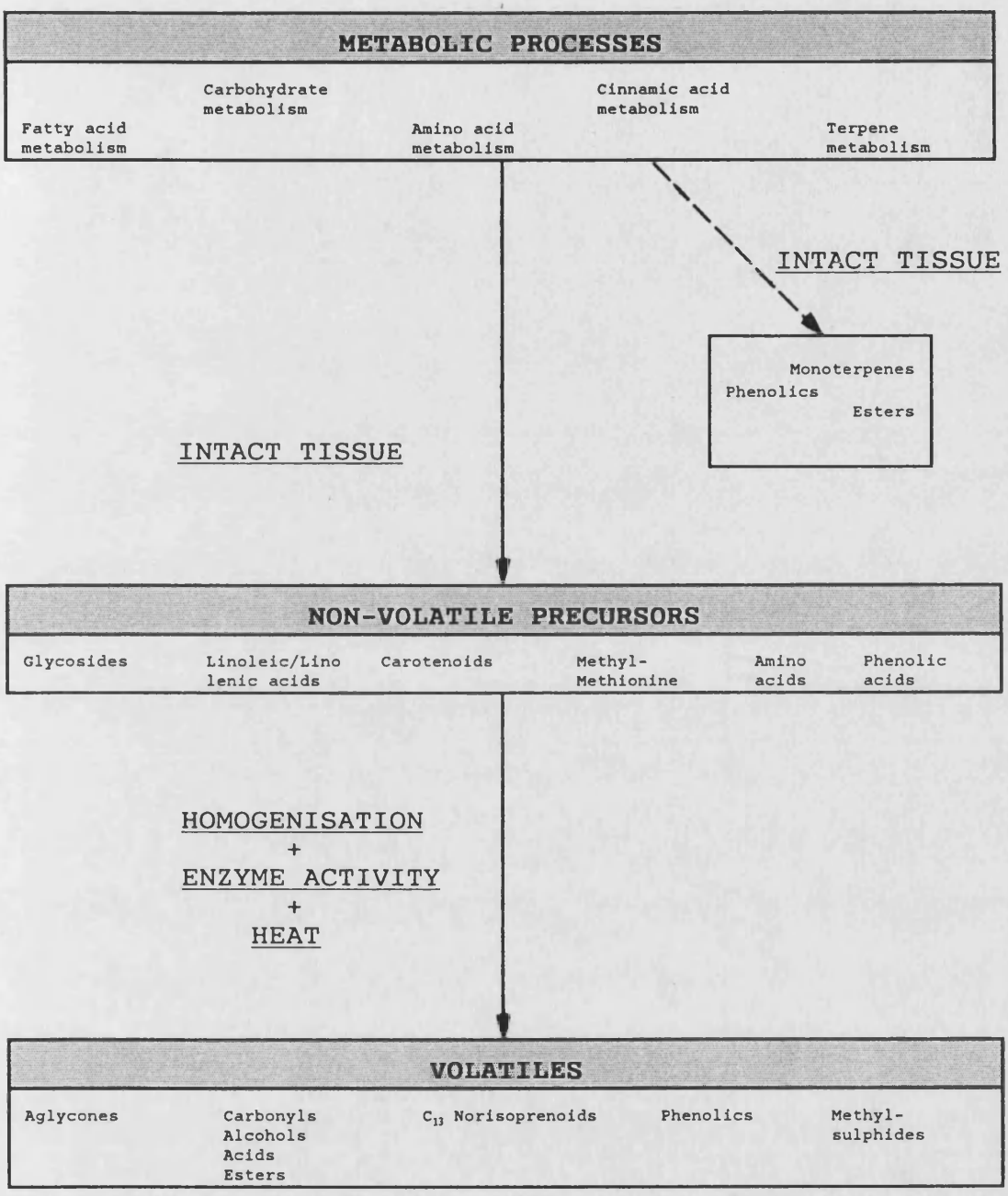
1) **Comparison of the cold dichloromethane, distilled and preheated rhubarb extracts**

Observation of the G.C. traces of the rhubarb samples (Figs D.1, D.2, D.3) reveals that the number of components increased in proportion to the degree of heating. Rhubarb is generally consumed cooked and organoleptic assessment confirmed that flavour approval was directly related to the degree of cooking. The cold dichloromethane extract had a sharp, astringent and earthy character, while the distilled sample was soft, fruity and sulphur like. The effect of preheating was to increase fruity and sulphurous notes while adding jammy and caramellic characteristics.

The components of the cold dichloromethane extract will be taken as being representative of A) Volatiles present in intact rhubarb, whereas the difference between these components and those in the heated samples will illustrate B) The rhubarb volatiles which develop during homogenisation and heating.

Fig.F.1 gives a general overview of volatile formation in rhubarb.

Fig.F.1 Proposed route for the synthesis and secondary formation of volatiles in Rhubarb



A) Volatiles present in intact rhubarb

76 volatiles were detected in the cold dichloromethane extract of rhubarb stalk (Table IV.1). This method of sample preparation was designed to limit the amount of secondary artifact formation. Homogenisation with solvent promoted denaturation of enzymes and increased the solvation of precursors away from any enzyme systems remaining active. Similarly, the immediate removal of volatiles from the highly acidic rhubarb homogenate helped to reduce acid-catalysed rearrangements. Low extraction temperatures further suppressed such secondary reactions. However, tissue breakage during pulling and leaf removal does inevitably expose the stalk to some oxidation and so it is not surprising that secondary volatiles were identified in the cold dichloromethane extract.

In 1975 Tressl *et al.*[21] suggested that only non-volatiles were present in intact vegetables and that these precursors were compartmentalised away from the enzymes necessary for their transformation into volatiles. It was proposed that homogenisation was able to initiate catabolism simply by the physical 'bringing together' of substrate and enzyme. Later researchers, while broadly agreeing with this, have recognised some flavour substances that are synthesised in intact vegetables: 2-isobutylthiazole in tomato, 2-methoxy-3-isobutylpyrazine in several vegetables,[22][23] and terpenes in herbs.[24]

Although the components characterised in the rhubarb samples follow Tressl's general principle (see the major reaction route in Fig.F.1), there is some evidence of the following groups of volatiles being present in intact rhubarb: monoterpenes, phenolics and shikimate derivatives, esters, alcohols and carbonyls, fatty acids and aromatic hydrocarbons.

Monoterpenes

The presence of nine monoterpenes in rhubarb is consistent with the apparently ubiquitous nature of these C_{10} volatiles in higher plants.[25] Monoterpenes do not constitute a major part of the volatile fraction of rhubarb, indeed observation of the g.c. trace of the dichloromethane extract reveals that only limonene and linalool are present in significant amounts.

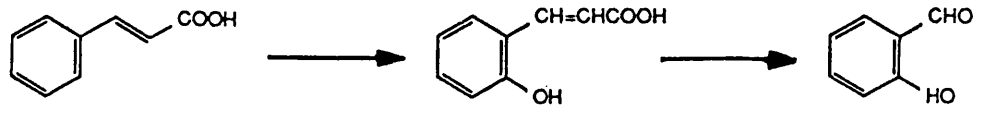
It is also noteworthy that with a pH of 3.5, acid-mediated interconversions (as elaborated in the discussion on homogenised and heated rhubarb samples) are probable even during extraction with cold dichloromethane. It can be concluded therefore that very few free monoterpenes, possibly only limonene and linalool, are present in intact rhubarb. Monoterpene glycosides, partially hydrolysed at pH 3.5, may also act as an alternative source of the traces of monoterpenes observed. Ten such glycosides have been identified so far in rhubarb.

Phenolics and shikimate derivatives

Few phenolic and shikimate-derived volatiles were found in intact rhubarb, although phenol, benzaldehyde and benzyl alcohol, very common derivatives in plants, were present. The trace of anisaldehyde observed may have been generated from its glycoside, a known component of rhubarb stalk, leaf and root. [Chapter 7]

Salicylaldehyde occurs in a range of foods including alcoholic beverages, vanilla, grape, cinnamon and tomato.[24] It is believed to arise from the shikimic acid pathway *via* cinnamic acid and *o*-coumaric acid.[26] Fig.F.2 summarises this.

Fig.F.2 Possible mechanism for the biosynthesis of salicylaldehyde in rhubarb



cinnamic Acid *o*-coumaric acid salicylaldehyde

Esters

Ethyl acetate, methyl butyrate, butyl acetate and 3-methylbutyl acetate were identified in the cold dichloromethane extract and so may be assumed to be present in intact rhubarb stalk. These findings are unusual as esters have rarely been observed in other vegetables. A few, such as *cis*-hexenyl acetate have occasionally been characterised (cabbage and watercress), whereas some herbs are known to contain esters of terpene alcohols. Only in Lambs lettuce has the occurrence of a range of esters (21) suggested active biosynthesis.[24] This difference may explain the more 'fruity' character of rhubarb and hence the reason why it is generally accepted as a dessert.

The majority of work investigating ester biosynthesis has been conducted on fruit such as tomato[22], banana[27] and musk melon[28]. Figs F.3 and F.4 show the pathways by which esters are synthesised from amino acids and fatty acids.

Fig.F.3 Pathway of ester synthesis from amino acids

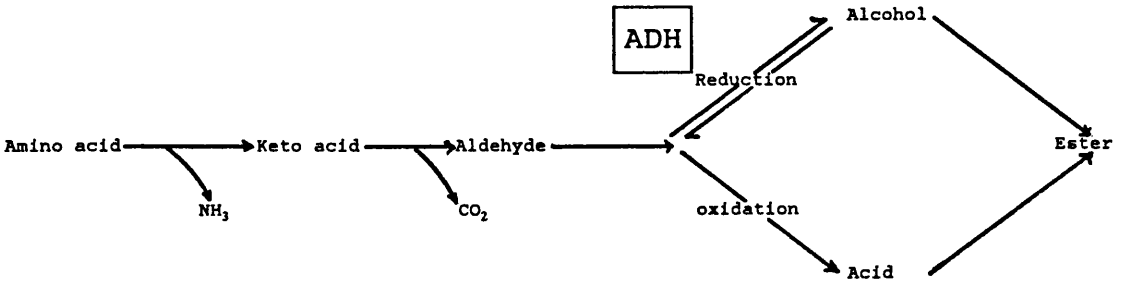
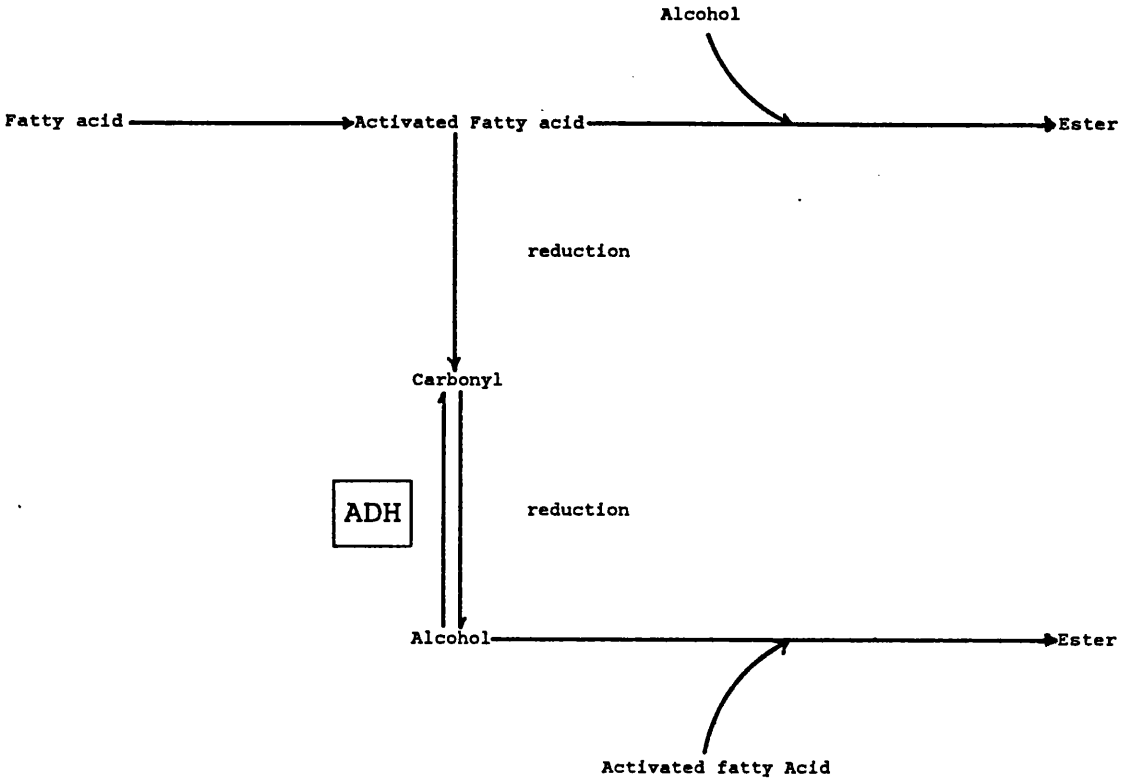


Fig.F.4 Pathway of ester synthesis from fatty acids



Both mechanisms require intact tissue. Trials with ^{14}C-labelled precursors are needed to confirm whether either ester synthesis was active or prevalent in rhubarb stalk.

Alcohols and carbonyls

In intact rhubarb the majority of alcohols and carbonyls identified are C_6 or smaller. Fig.F.3 shows, schematically, how plants can convert some amino acids into volatiles. In this way leucine, valine and phenylalanine have been characterised as the precursors of 3-methylbutanol, 2-

methylpropanol and 2-phenylethanol respectively.

Researchers have frequently observed the presence of carbonyl/alcohol pairs (such as hexanal/hexanol) in plants[29][30] and attributed this to the activity of oxidoreductases such as alcohol dehydrogenase (ADH). Related pairs occurring in rhubarb are 3-penten-2-ol/3-penten-2-one, octanol/octanal and octen-3-ol/octen-3-one. Trials involving the addition of [^{14}C] labelled amino acids to banana and tomato, have revealed that although the reaction catalysed by ADH is reversible, the equilibrium is almost exclusively towards the alcohol[27][31]. The presence of 3-methylbutanol, but not the aldehyde, in rhubarb may therefore be the result of an equivalent ADH acting on leucine metabolites. The trace of 2-phenylethanol detected indicates that phenylalanine is similarly catabolised in the stalks.

In parallel trials, [^{14}C] labelled saturated fatty acids were found to be reduced to their equivalent alcohols[27]. As before, ADH in fruit favoured alcohol formation however, in intact rhubarb, the co-occurrence of octanal seems to suggest that the ADH involved in octanoic acid catabolism is somewhat less biased towards the alcohol.

C_5 and lower carbonyls and alcohols prevalent in rhubarb (2-butenal, 3-methyl-3-buten-2-one, 2-methyl-3-buten-2-ol, 3-penten-2-one, 3-penten-2-ol, 2-methyl-2-butenal and 2-

methyl-2-butenol) also occur variously in tomato[32], blackcurrant[33], cherimoya[34], cape gooseberry[35] and peach[36]. These volatiles are similar in structure to both mevalonic acid and isoprene units (precursors of terpenoids in plants[35]) and may either be derived from these compounds or be involved in terpene biosynthesis themselves. Unlike the fruits listed above, rhubarb contains relatively few terpenes and so it would appear that, despite the significant quantities of these C_5 precursors, conversion to monoterpenes is rather limited.

3-penten-2-one readily forms when linolenic acid is photooxidised[37] and its generation from this source during sample preparation cannot be ruled out.

Fatty Acids

It is known that free fatty acids are absent in healthy, intact plant tissue, accumulating instead in the form of lipids[38]. Phospho- and galacto-lipids constitute the majority of these and are believed to be components of compartmentalising membranes in plant tissue. One such example is the thylakoid membrane of chloroplasts.

Trials with tomato and cucumber lipids[38][39] have shown that homogenisation can cause enzyme-catalysed deacylation, with the consequence that fatty acids are released. In this way high levels of palmitic, linoleic and linolenic acids can

form. In rhubarb, the cold dichloromethane extract contained quantities of these higher acids, suggesting lipid degradation had, in part, occurred. The pH optimum of the tomato enzymes was found to be 3.5 which is also the approximate pH of rhubarb stalk.

In intact mitochondria, palmitic acid can be cleaved by β-oxidation to give lower straight-chained fatty acids and this perhaps explains the additional identification of C_{14}, C_{12}, C_{10} and C_8 acids in rhubarb stalk. The absence of lower fatty acids reveals that further β-oxidation was curtailed in the cold dichloromethane extract, probably because of solvent-mediated enzyme denaturation.

Aromatic hydrocarbons

Although aromatic hydrocarbons, including toluene, xylene, styrene, trimethylbenzene and naphthalene are present in all of the rhubarb extracts, the majority are found after cold dichloromethane extraction. This confirms work involving heated and raw blackcurrants and nectarines[33][40], where vacuum distillation caused the loss of all the aromatic hydrocarbons previously identified by direct solvent extraction. This is perhaps surprising in view of the experiments of Seck and Couzet[41] who observed the development of aromatic hydrocarbons when solutions of phenylalanine were heated. In spite of this there does appear to be a clear trend, to which rhubarb conforms,

suggesting that aromatic hydrocarbons are particularly prevalent in raw fruit and vegetables.

B) The rhubarb volatiles which develop during homogenisation and heating

A large number of volatiles develop in rhubarb as a result of homogenisation and heating. These volatiles have been classified into the following groups: monoterpenes, sesquiterpenes, phenolics and shikimate derivatives, aldehydes, alcohols, carboxylic acids, lactones, esters, furans, Strecker degradation products, other amino acid derived volatiles and carotenoid-derived products. The volatiles derived from linoleic and linolenic acids are covered in greater detail because of their apparent importance to rhubarb flavour. Possible mechanisms for the presence of the flavour components are discussed in each section.

Table VI.1

Monoterpenes detected in rhubarb extracts

	RHUBARB EXTRACT		
MONOTERPENES	1	2	3
1,4-Cineol	-	+	-
α-Pinene	+	-	-
Sabinene	+	-	-
p-Cymene	+	+	+
Limonene	+	+	+
γ-Terpinene	+	-	-
trans-Linalool-oxide (furan)	-	-	+
cis-Linalool-oxide (furan)	-	+	+
Linalool	+	+	+
β-Terpineol	-	+	+
Menthol	+	+	+
Terpinen-4-ol	+	+	+
α-Terpineol	-	+	+
Carvone	+	+	-
Geranial/Neral	-	+	-

Key

1: Cold dichloromethane extract

2: Distilled sample

3: Preheated sample

Monoterpenes

Table VI.1 lists the monoterpenes present in the three rhubarb extracts. α-Pinene, sabinene and γ-terpinene, trace components of the cold dichloromethane extract, were not detected in samples 2 and 3. This might be due to their coelution with thermally generated compounds or they may simply breakdown, rather in the manner of the terpene decomposition reported in heated blackcurrant[33]. In spite of this, most researchers have either observed little lysis of terpenes[42][43][44] or have proposed mechanisms for the thermally induced conversion of one terpene to another, e.g. linalool to α-terpineol (see later). Care must be taken with the results of some workers[45], which, although initially indicating that thermal breakdown had taken place, revealed on closer examination that loss of terpenes was more probably due to evaporation from open vessels.

Observation of the GC trace of each rhubarb sample (Fig.D.1, D.2 and D.3) reveals that the increase in α-terpineol level is directly proportional to the degree of heating. Similar effects are seen with blackcurrant[33], apricot[42] and tomato[44]. Linalool, on the other hand, appears to decrease during heating of rhubarb and research with solutions of this compound has suggested that mild heating leads to its conversion to α-terpineol[154]. The reduced levels of linalool may also be attributable to the formation of linalool-oxides *via* oxidation in the distilled

and preheated rhubarb samples. Linalool-oxides may also be released from glycosides during heating. The general formation of linalool-oxides is discussed further in Chapter 7.

Schieberle and Grosch analysed acidified solutions of lemon oil, stored at 37°C, and revealed a pattern of terpene breakdown which included the loss of neral and geranial and the formation of *p*-cymene,terpinen-4-ol,*p*-cresol and 4-methylacetophenone[46]. Although *p*-cymene and terpinen-4-ol were identified in all the rhubarb samples, levels were highest in the preheated and distilled extracts. *p*-Cresol was not detected in any of the samples. 4-Methylacetophenone, however, was a component of distilled rhubarb and is also known to form when geranial-containing blackcurrant juice is heated[33]. From these results it can be concluded that, during heating, degradation of neral and geranial originally derived from carotenoids (see later), leads to the formation of some of these volatiles. *p*-Cymene, terpinen-4-ol and 4-methylacetophenone are described, respectively, as gasoline, musty and bitter-almond like in character[46] and probably contribute to the more complex aroma of the heated rhubarb extracts.

1,4-Cineol, which occurred in the distilled sample, was most likely released from glycosides present in the rhubarb stalks. 1,4-Cineol has also been identified in heated blackberry juice[47].

Sesquiterpenes

Sesquiterpenes and sesquiterpene alcohols were only tentatively identified in rhubarb as no standards were available to provide comparative retention-time data. Sesquiterpenes also give extremely similar mass spectral fragmentation patterns, making matching with library data of limited value. It can be seen, however, that there are numerically more sesquiterpenes in the heated samples of rhubarb - especially the distilled extract. This is probably due to the acid-catalysed interconversion of sesquiterpenes during heating. It is possible that the presence of these different sesquiterpenes will have an effect on the flavour of cooked rhubarb.

Phenolics/Shikimate derivatives

The formation of phenolic volatiles during heating has been shown by several researchers to be the result of thermal decarboxylation of phenolic acids[34][49][158]. Applying this to the rhubarb extracts, it may be postulated that vanillin originates from ferulic acid, 4-allylphenol from coumaric acid and syringaldehyde from sinapic acid. Alternatively they may be released from glycosides by hydrolysis, along with volatiles such as 2-phenylethanol. The mechanisms by

which 1-methoxy-4-methylbenzene and eugenol methyl ether (4-allyl-1,2-dimethoxybenzene) are evolved are not known. Neither have previously been reported to form as a result of homogenisation and/or heating. A more detailed pathway for ferulic acid decarboxylation is shown in Chapter 7.

Aldehydes

There is evidence of increased levels of aldehydes in the distilled and preheated rhubarb extracts as compared to the cold dichloromethane extractive. The formation of aldehydes from linoleic, linolenic and amino acids is discussed later while the presence in rhubarb of benzaldehyde and anisaldehyde, bound as glycosides, is reviewed in Chapter 7. It would seem likely that glycoside hydrolysis causes the release and subsequent detection of higher levels of these two aldehydes in the heated samples.

Two further precursors of aldehydes in rhubarb may be oleic and stearic acids. Although free oleic acid has not been identified, it is present in the triglycerides of many plants. Hydrolysis of such a triglyceride in rhubarb may yield the free acid which, on thermal oxidation, can liberate heptanal, octanal, nonanal, decanal, *trans*-2-decenal and *trans*-2-undecenal[50], all components of the distilled and preheated extracts. Similar breakdown has

been characterised in heated potato[51], soyabean[29] and rice bran[52][53]. The equivalent high temperature lysis of stearic acid evolves hexanal, heptanal and octanal. However, since these aldehydes may also be formed by other mechanisms their presence does not provide conclusive evidence for stearic and oleic acid decomposition.

Alcohols

The majority of alcohols present in the rhubarb extracts are products of unsaturated fatty acid degradation and as such are discussed later. However, other alcohols such as 2-ethylhexanol, 2-(2-butoxyethoxy)ethanol and decanol also appear, or increase significantly, in the distilled and preheated extracts.

Decanol is known to form when higher saturated fats and fatty acids are heated and oxidised at temperatures >60°C[54]. An example in rhubarb might be stearic acid or its triglyceride which, at the temperature of the preheated extract, could break down to give decanol[50]. This may also be the source of increased octanol levels observed in the preheated GC trace.

2-Ethylhexanol and 2-(2-butoxyethoxy)ethanol are shown to be bound as glycosides in rhubarb stalk and leaf (Chapter 7). Heating may cause the breakdown of these glycosides in the distilled and preheated rhubarb samples. There is a similar increase of 2-ethylhexanol in heated blackberry juice[47] and 2-(2-butoxyethoxy)ethanol in strawberry jam[24] but, as yet, neither alcohol has been identified as a glycoside in these fruit[55]. 2-Ethylhexanol has been used as a plasticiser[56] and as such, may be a contaminant.

Carboxylic acids

The homogenisation and heating of various food systems is known to increase the level of carboxylic acids. Table VI.2 reveals that this also occurs when rhubarb stalk is liquidised and cooked. As mentioned earlier, various workers have shown that when triglycerides are heated in high moisture systems such as rhubarb, the ester linkage is hydrolysed and carboxylic acids released[50][155]. Elevated temperatures can also lead to the rapid oxidation of alcohols and aldehydes to their corresponding acids[50][57]. Hence the C_6-C_{10} alcohols and their respective aldehydes could all act as precursors of the carboxylic acids present in the heated samples. Hydrolysis of triglycerides in rhubarb may also be catalysed enzymatically either by

endogenous lipases/esterases or by enzymes produced by yeasts and moulds present on the surface of the stalks. Activity would continue until heat, or the addition of dichloromethane, denatured the enzymes.

Table VI.2

Carboxylic acids identified in rhubarb

ACID	RHUBARB EXTRACTS		
	1	2	3
Acetic acid	-	+	+
Hexanoic acid	-	trc	+
Heptanoic acid	-	-	+
Octanoic acid	-	trc	+
Nonanoic acid	trc	+	+
Decanoic acid	trc	+	+
Dodecanoic acid	+	+	+
Tetradecanoic acid	+	+	+
Hexadecanoic acid	+	+	+
Linoleic acid	+	+	+
Linolenic acid	+	+	+
Chrysophanic acid	-	-	+

Key

1: Cold dichloromethane extract

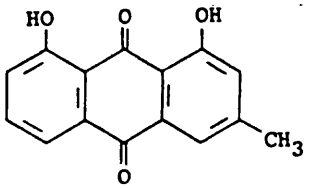
2: Distilled sample

3: Preheated sample

Triglycerides may not be the only source of carboxylic acids in rhubarb. Large amounts of hexanoic acid were found in glucose/glutamic acid browning systems although the mechanism of formation was unknown[59]. Acetic acid is also well documented as a common product of glucose degradation[60].

Chrysophanic acid was identified in Chinese rhubarb root[9] and has been known as a component of edible rhubarb for many years[58][3]. The process of preheating appears to have increased its concentration such that it was identifiable by gas chromatography - see Fig.D.3. Chrysophanic acid was not released as an aglycone when glycosidic fractions of rhubarb root, stalk and leaf were enzymatically hydrolysed (see Chapter 5). Other workers however, observing the intact glycosides of chinese rhubarb, were able to purify chrysophanic acid bound to glucose[16]. This shows perhaps, that the hot/acidic conditions of preheated and homogenised rhubarb were more active in hydrolysing some glycosides than were exogenous β-glucosidase and macerase enzymes.

Chrysophanic acid



Lactones

Lactones are uncommon in rhubarb with only three (γ-hexalactone, *d*-nonalactone and γ-decalactone) being identified in the preheated sample. Here the greater degree of heating may encourage the chemical formation of lactones. This effect has been observed in several food systems including tea[62], rice[53][61], beef fat[62][63], milk products[62], heated glucose/glutamic acid[59], blackberry[47] and plum[45]. Lower lactones such as γ-hexalactone have generally formed in the heated fruit, while higher lactones (e.g. γ-decalactone) tended to appear in high-fat systems. Various mechanisms have been proposed for their presence including the cyclisation of hydroxy-acids released from triglycerides[64] and the autoxidation/cyclisation of unsaturated and saturated fatty acids[63][62]. The latter is possible in rhubarb where fatty acids are abundant. γ-Hexalactone has been described as herbaceous and sweet in character, *d*-nonalactone as fatty and nutlike and γ-decalactone as fruity. An alternative formation mechanism was alluded to by Takeoka *et al.*[57] who observed the formation of ten *d*-lactone artifacts during the three-month frozen storage of a solvent extract of kiwi fruit. No hypothesis was suggested to explain their formation, although the low water content of the extract may have been significant. The same effect might occur in the similarly water-free preheated rhubarb extract, but does not explain

why other rhubarb samples were unaffected.

Esters

Table VI.3 compares the esters identified in various rhubarb extracts and clearly shows that there are significantly more esters present in the samples that had been homogenised and heated. The formation of esters in intact rhubarb has been discussed earlier in relation to the volatiles detected in the cold dichloromethane extract.

The effect of homogenisation and heating on ester levels has been studied in many food systems. Very different results have been observed and these will be summarised and related to rhubarb in three sections:-

i) Ester formation

ii) Ester formation in concentrated extracts

iii) Ester breakdown.

Table VI.3 Esters identified in Rhubarb Samples

DCM extracted Rhubarb	Distilled Rhubarb	Preheated Rhubarb
Ethyl acetate	Ethyl acetate	Ethyl acetate
Methyl butyrate	Methyl butyrate	ND
ND	Ethyl butyrate	ND
Butyl acetate	Butyl acetate	ND
3-Methylbutyl acetate	3-Methylbutyl acetate	3-Methylbutyl acetate
Methyl hexanoate	Methyl hexanoate	ND
ND	Ethyl hexanoate	ND
ND	Hexyl acetate	ND
ND	Phenylethyl acetate	ND
ND	ND	Methyl 3-furoate
ND	Menthyl acetate	Menthyl acetate
ND	Ester I	Ester I
ND	Ester II	Ester II
ND	Geranyl acetate	ND
ND	ND	2-Methylpropyl decanoate

DCM extracted Rhubarb	Distilled Rhubarb	Preheated Rhubarb
ND	Diester	Diester
ND	ND	2-Methylpropyl tetradecanoate

Key

ND = Not Detected

Ester I = 2,2,4-Trimethyl-1,3-pentanediol-2-methylpropanoic acid ester (1-hydroxy unbound)

Ester II = 2,2,4-Trimethyl-1,3-pentanediol-2-methylpropanoic acid ester (3-hydroxy unbound)

Diester = 2,2,4-Trimethyl-1,3-pentanediol di-2-methylpropanoic acid ester

i) Ester formation

Table VI.3 reveals that distilled and preheated rhubarb contain increased levels of those esters with mass higher than that of methyl hexanoate. This may be due to either enzymatic ester synthesis or heat-induced chemical synthesis in the homogenised stalk. Work with uncooked and cooked rice[61][53] has suggested that heating alone could be responsible for the formation of these higher esters. Alternatively, Schen *et al.*[65] proposed an enzyme-catalysed formation of higher esters in strawberry pomace stored at 40°C/pH4. The fact that esters lower than methyl hexanoate were greatly reduced at the same time, was thought to be because the enzyme system favoured higher esters. Clearly there are similarities here with the rhubarb results outlined in Table VI.3.

During the analysis of cape gooseberry volatiles Berger *et al.*[35] found that, when a high level of alcohol solvent was added to homogenised fruit, activated acyl (acid) moieties in the fruit were able to form `secondary` esters with the alcohol. They further suggested that on homogenisation these activated acyl moieties were also able to esterify endogenous alcohols and those alcohols naturally formed after tissue breakage. The character of the acyl activation was not known but did apparently lead to the formation of many of the esters detected. It might be speculated that similarly activated acyl moieties may be

present in rhubarb and are able to be esterified after homogenisation and heating.

ii) Ester formation in concentrated extracts

The concentrated trichlorofluoromethane extracts of kiwi fruit discussed in the section on lactones also formed hexyl formate and hexyl hexanoate after storage at -20°C[57]. It appeared that in the low-water environment hexanol was non-enzymatically esterified. Similarly, when a must containing very few esters was distilled in order to produce a [52% ethanol] apple brandy, many ethyl esters were formed[67]. Again, ethanol was probably non-enzymatically esterified in the less aqueous conditions. Higher esters may form in a similar manner during (frozen) storage of the dried rhubarb extracts e.g. 2-methylpropyl decanoate and tetradecanoate in the preheated sample. These have previously been identified in grape brandies, fruit brandies and whiskies[24], all with reduced water contents.

iii) Ester breakdown

Esters which were already present in the cold dichloromethane extract of rhubarb remained intact in the distilled sample, but were mostly lost when the rhubarb was preheated. This loss of esters can be attributed either to

the activity of enzymes or to the process of heating. However, as is shown below, esters appear to be affected in vastly different ways in various systems.

There was total loss of esters when Reine-Claude plums were heated for 4 hrs[45] while pasteurisation of guava puree (85°C for 24 secs) was sufficient to significantly reduce all the esters present[66]. In contrast to this, distillation of tomato puree and the autoclaving of strawberry puree at 120°C did not apparently hydrolyse esters (including exogenous esters) in either system[156]. Another analysis of heated tomato revealed that only linoleate and linolenate esters were broken down[43], possibly because of the lability of the acid moiety rather than the ester linkage.

Carboxy-esterases have been implicated in ester hydrolysis during apple mashing[67], their activity being greatly influenced by pH and temperature[68]. Conversely, the loss of esters in homogenised Mirabelle plums[69] was attributed to the denaturation and inhibition of enzymes involved in ester biosynthesis, with esters already present presumably being hydrolysed. A similar enzyme denaturation has been demonstrated in homogenised strawberry and banana[70][65].

In conclusion, it would appear most likely that the lower esters are lost from the preheated rhubarb sample by thermal hydrolysis. The formation of higher esters in the

distilled and preheated extracts, although not commonly encountered elsewhere, is most probably either enzymatic or thermal in origin. Alternatively, these esters may form during the storage of the concentrated extracts.

There are three further esters of particular interest in the heated rhubarb samples (ester I, ester II and diester in Table VI.3). These esters are composed of trimethylpentanediol and 2-methylpropanoic acid and were detected in all samples of rhubarb stalk, except the cold dichloromethane extract. The esters have not been found in rhubarb root and the free diol was not identified in any rhubarb tissue. Previously, the diester has been found in french-fried potatoes[24] and ester II in hontohroku and taishokintoki beans (raw)[24]. Combinations of ester I, ester II and the diester have been used as plasticisers in PVC films and non-stain floor coverings[71]. Kim *et al.*[56] identified ester II as a minor food volatile which had been able to migrate from PVC packaging. If the esters do not actually originate from the rhubarb tissue it is likely that they are due to contamination, perhaps from the plastic bags in which the stalks were frozen. However, this does not explain the absence of these esters in similarly stored roots (unpublished data) or their presence in the canned rhubarb extract.

Furans

Furans are well documented as products in heated foods and are believed to derive from carbohydrate dehydration. Furfural, which was found in the preheated rhubarb and at trace levels in the distilled sample, is known to form in model systems where fructose, glucose and sucrose are heated at acid pH[41][72]. Some workers have also characterised cellulose[73] and pectic substances[41] as sources of furfural when heated. 5-Hydroxymethylfurfural constituted 80% of the aroma extract of cooked plum and was considered as evidence of sugar degradation[45]. The addition of sucrose to blackberries prior to heating was found to increase the level of this furfural five-fold, whilst other furans were unaffected[47]. Interestingly, blackberry varieties that did not inherently form 5-hydroxymethylfurfural were not induced to after the addition of extra sucrose. Therefore, to form this furfural, it seemed both sucrose and another component present only in some blackberry varieties, were required. Rhubarb forms much lower levels of 5-hydroxymethylfurfural during heating than does blackberry or plum. This may be connected to its lower sugar content in relation to these fruit, nevertheless the same general mechanism of formation would appear to apply.

Methyl furoate has been detected in several heated foods including blackberries[47] and plums[45], while linalool-oxide (furan) can form *via* several processes (see Chapter 7).

In general, these furans exhibit caramellic, jammy and cooked aromas which add a pleasant note to the character of heated rhubarb.

Strecker degradation products

Strecker degradation is the stage of the Maillard reaction in which amino acids are converted to volatile aldehydes[41]. Table VI.4 compares the various amino acids, their respective Strecker aldehydes and those present in rhubarb, while Fig.F.5 shows the degradation mechanism for methionine.

Strecker aldehydes can form in many heated food systems including potato (baked[74][56], dehydrated[72], boiled[72]), globe artichoke[75], tomato[76][32], blackcurrant[47], plum[45] and meat[159]. In some cases, heating at 90°C for five minutes was sufficient to produce significant levels of these breakdown products. Tasting trials by many workers have revealed a strong link between the presence of these aldehydes and the perception of heated and cooked notes. Table VI.4 demonstrates the necessity for heat in Strecker aldehyde production, but probably underestimates their number in rhubarb since acetaldehyde and 2-methylpropanal are likely to coelute with the solvent. Head-space analysis of samples might confirm the presence of these more volatile aldehydes.

Benzaldehyde is also included in Table VI.4 as many workers have assigned it as a breakdown product of phenylacetaldehyde[41][33], itself derived from phenylalanine. Benzaldehyde could also be an aglycone released from glycosides present in the rhubarb stalk (see Chapter 7).

Table VI.4

Strecker aldehydes, their precursor amino acids and their detection in rhubarb samples

		RHUBARB SAMPLE		
ALDEHYDE	AMINO ACID	1	2	3
Acetaldehyde	Alanine	-	-	-
2-Methylpropanal	Valine	-	-	-
3-Methylbutanal	Leucine	-	+	-
2-Methylbutanal	Isoleucine	-	-	-
Phenylacetaldehyde	Phenylalanine	-	+	+
Benzaldehyde	Phenylalanine	-	+	+
Methional	Methionine	-	+	+

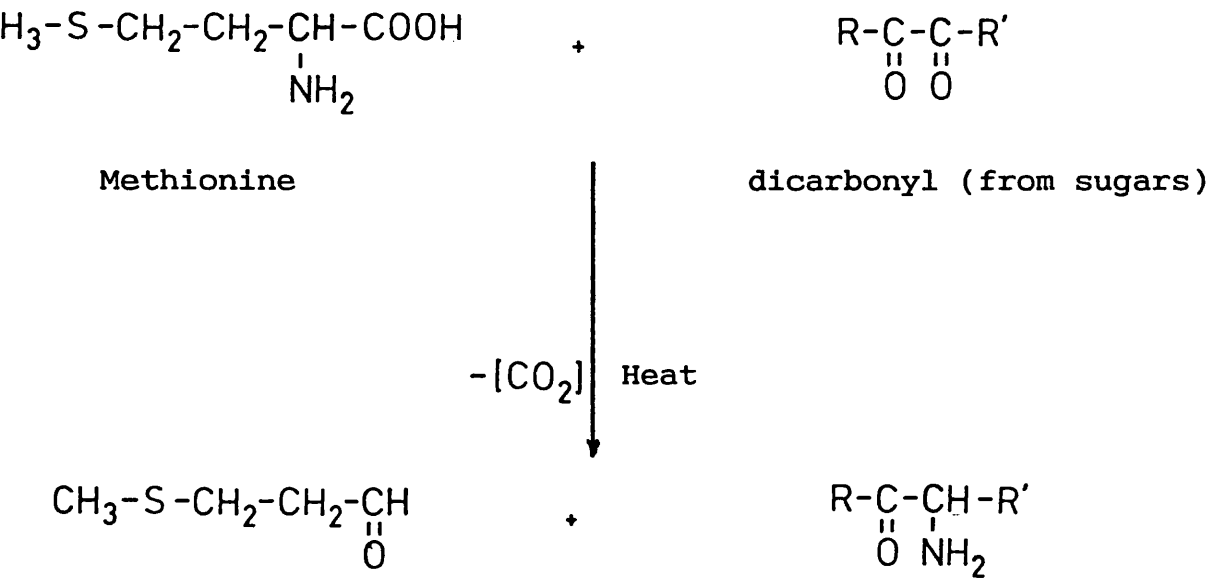
key

1: Cold dichloromethane extract

2: Distilled sample

3: Preheated sample

Fig.F.5 The Strecker degradation of Methionine



Other amino acid derived volatiles

A cursory appraisal of the aroma of the three rhubarb stalk extracts reveals the presence of sulphur containing components (other than Strecker aldehydes) in the heated samples. The aroma is described as cabbage or heated tomato in character and is typical of sulphides such as dimethylsulphide and dimethyldisulphide. Although these sulphides could not be searched for in this study, previous workers have suggested mechanisms for their production[21]. The intact tissues of vegetables are believed to contain non-volatile precursors some of which may be chemically hydrolysed during heating[43][76]. Examples of these

precursors include methylmethionine sulphonium salts, methylcysteine and methionine, all of which can decompose on heating to give, variously, dimethyl sulphide, methanethiol and dimethyl disulphide. As has been mentioned, the aroma of heated rhubarb suggests that some of these volatiles may have been formed.

The heating of amino acids, with or without sugars, also encourages the formation of nitrogen-containing heterocyclic compounds such as pyrazines and pyrroles[77]. These compounds have burnt, toasted and nutty characters and are formed from the thermal degradation of precursors under relatively dry conditions. It is unsurprising therefore, that pyrazines are major components of the skin of baked potato but not of the moist centre[56]. However, during the heating of rhubarb, all samples remained moist and, as might be anticipated, no pyrazines and only one pyrrole were identified. This agrees with most other wet plant materials which have been heated[76] as well as the results achieved with model, aqueous systems[41]. Pyrrole has previously been identified in cooked foods such as crispbread, boiled egg, chicken, beef, pork, cocoa and coffee[24].

Benzothiazole[24], was found in both heated rhubarb samples and has also been identified in the following heated products: potato, asparagus, leek, crispbread, coffee, cocoa, filbert and peanut. Berry and Gramshaw[59] also

detected the compound in a dry-heated glucose-glutamic acid system declaring it was not easy to account for its formation by either the Maillard reaction or sugar caramelization. It is difficult to say whether benzothiazole formed in rhubarb as a result of heating, for this compound has also been detected in raw apple, apricot, guava, asparagus, nectarines etc.[24].

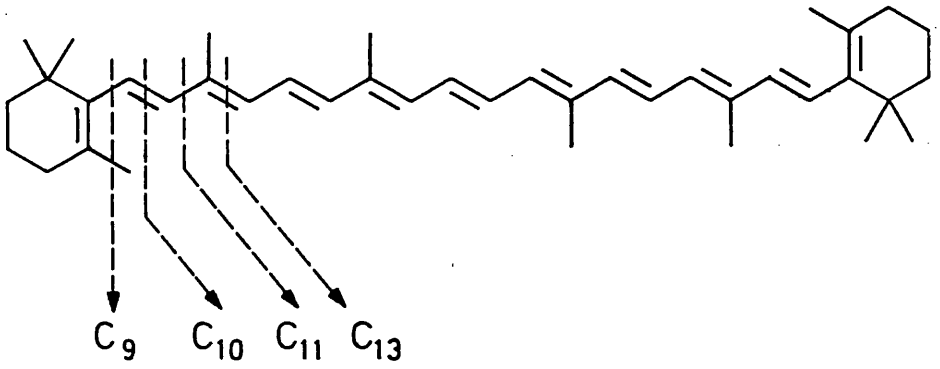
Carotenoid-derived products

Carotenoids occur in practically all plants and are biosynthesised in chloroplasts by a series of condensation, rearrangement and cyclisation reactions[26][81]. They act as a potent source of flavour and aroma compounds.

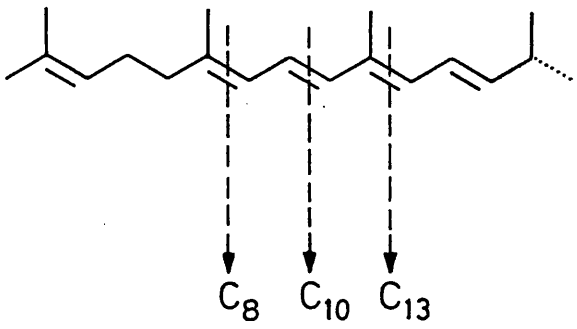
Fig.F.6 shows the site of breakdown and primary oxidation of three common carotenoids. Table VI.5 lists the volatiles detected by various workers during the oxidation of these carotenoids[80][82][83] and details those found in the three rhubarb extracts. Little or no carotenoid oxidation occurred in the cold dichloromethane extract whereas the others contained products consistent with the oxidation of all three common carotenoids. Many of these products have low organoleptic thresholds and may significantly affect the flavour of rhubarb. The two possible mechanisms of oxidation in the distilled and preheated rhubarb samples are i) Thermal and ii) Enzymatic in nature.

Fig.F.6

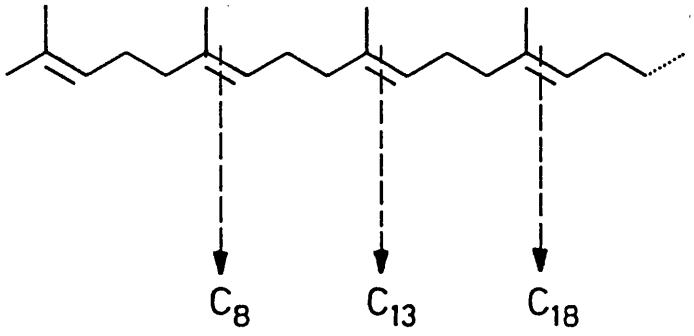
Oxidative cleavage of β-carotene[79]



Oxidative cleavage of *trans*-lycopene[80]



Oxidative cleavage of Solanesol[79]



Key

C_x = product contains x carbon atoms

Table VI.5

Comparison of Carotenoid oxidation products in standards and rhubarb samples

Volatile	Solanesol Solution	Lycopene Solution	β-Carotene Solution	Rhubarb Sample 1	2	3
6-Methyl-5-hepten-2-one	+	+	+	-	+	+
Geranylacetone	+	-	-	-	+	-
Farnesylacetone	+	-	-	-	-	-
5-Hexen-2-one	-	+	-	-	-	-

Hexane-2,5-dione	-	+	-	-	-	-
6-Methyl-3,5-heptadiene-2-one	-	+	-	-	-	-
Neral	-	+	-	-	+	-
Geranial	-	+	-	-	+	-
Geranyl acetate	-	+	-	-	+	-
Pseudoionone	-	+	-	-	-	-
2-Hydroxy-2,6,6-trimethylcyclohexanone	-	-	+	-	-	-
β-Cyclocitral	-	-	+	-	-	-
Naphthalene	-	-	+	-	-	+
β-Ionone	-	-	+	-	+	+
5,6-Epoxy-β-ionone	-	-	+	-	-	-
Dihydroactinidiolide	-	-	+	-	+	+

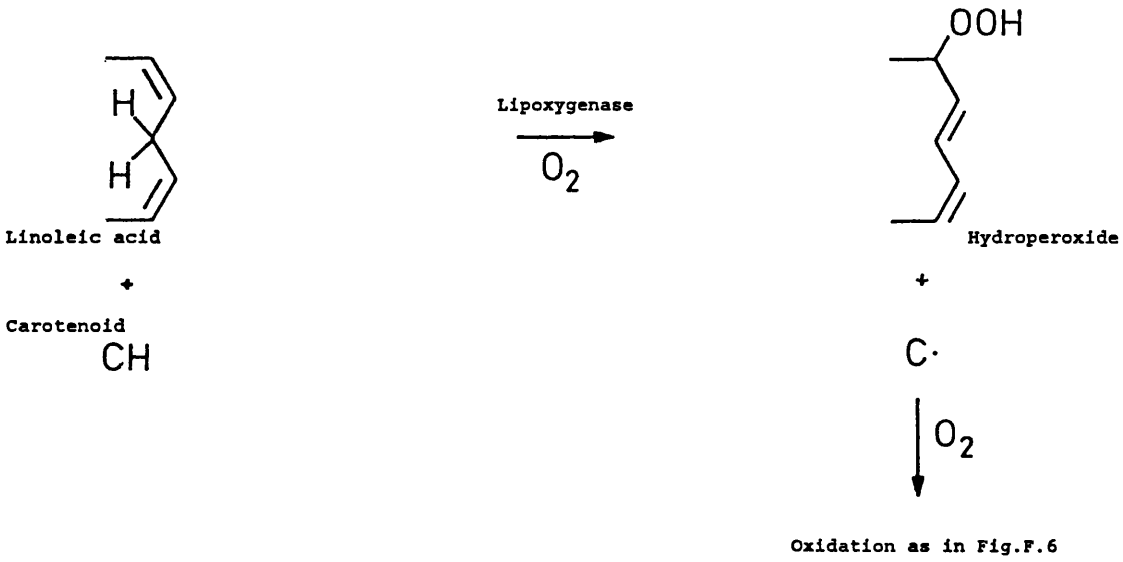
i) Thermal oxidation

Thermal oxidation of carotenoids has been observed in many systems such as cooked rice[53], cured tobacco[82], algae[84] and aqueous solutions of standard precursors[80][83][85]. Dihydroactinidiolide is probably the first volatile compound produced during heating of β-carotene, indeed, temperatures as low as 30°C have been sufficient to produce significant levels. Similar trials with *trans*-lycopene suggested that the double bond between carbon 6 and carbon 7 (leading to the formation of 6-methyl-5-hepten-2-one) is most sensitive to molecular oxygen during heating.

ii) Enzymatic co-oxidation

Lipoxygenase enzymes have been characterised in green plants and shown to oxidise unsaturated acids in the presence of air [see later]. Particular lipoxygenases (L-2 and L-3) have also shown a co-oxidation potential in which both unsaturated acids and carotenoids are oxidised simultaneously (Fig.F.7[82][86]).

Fig.F.7 The co-oxidation of unsaturated acids and carotenoids by lipoxygenases



This co-oxidation has been reported in homogenised endive[82] and has been implicated in the formation of carotenoid breakdown products in cape gooseberries[35] and tomatoes[44]. In the latter, intimate mixing of tomato enzymes, their substrates and air was thought to greatly favour the development of products formed by enzymatic co-oxidation. In rhubarb samples 2 and 3 both carotenoid and unsaturated fatty acid breakdown products have been found. It is highly likely therefore, that enzymatic co-oxidation proceeded in the distilled and preheated samples from the point of homogenisation until heating denatured the enzymes. Any further oxidation would then be thermal in nature.

Table VI.5 reveals the lower number of carotenoid oxidation

products in the more aggressively 'pre'heated rhubarb than in the distilled. An explanation of this effect is the further thermal breakdown of these oxidation products and hence their removal from the system. For example, neral and geranial can cyclise to form *p*-cymene and, when aqueous solutions of these terpenes are heated, their levels decline rapidly[87].

In conclusion, it seems likely that both β-carotene and *trans*-lycopene are available for oxidation in rhubarb stalk and can thus contribute to its flavour.

Linoleic and linolenic acid derivatives

Linoleic acid and linolenic acids were identified as components of all three rhubarb stalk extracts. Their oxidative decomposition appears to be a major source of flavour chemicals in rhubarb as well as many other foods. This discussion is separated into five sections:-

a) Formation of linoleic and linolenic acid hydroperoxides.
b) Breakdown of linoleic acid hydroperoxides.
c) Breakdown of linolenic acid hydroperoxides.
d) Secondary autoxidation of alkanals, alkenals and alkadienals.
e) Retro-aldol related degradation of alkenals and alkadienals.

These cover the general thermal breakdown mechanisms of these acids and their relevance to the flavour of rhubarb. Enzyme mediated oxidation is covered elsewhere.

a) Formation of linoleic and linolenic acid hydroperoxides.

The generally accepted free-radical mechanism for the autoxidation of linoleic and linolenic acids is shown in Fig.F.8[50][88].

Fig.F.8 Free-radical autoxidation of linoleic and linolenic acids.

Initiation

$$RH \xrightarrow{\text{initiator}} R.$$

(free radical)

Propagation*

$$R. + O_2 \rightleftharpoons ROO.$$

(peroxy radical)

$$ROO. + RH \longrightarrow ROOH + R.$$

(hydroperoxide)

Termination

$$ROO. + ROO. \longrightarrow \text{non-radical products}$$

key

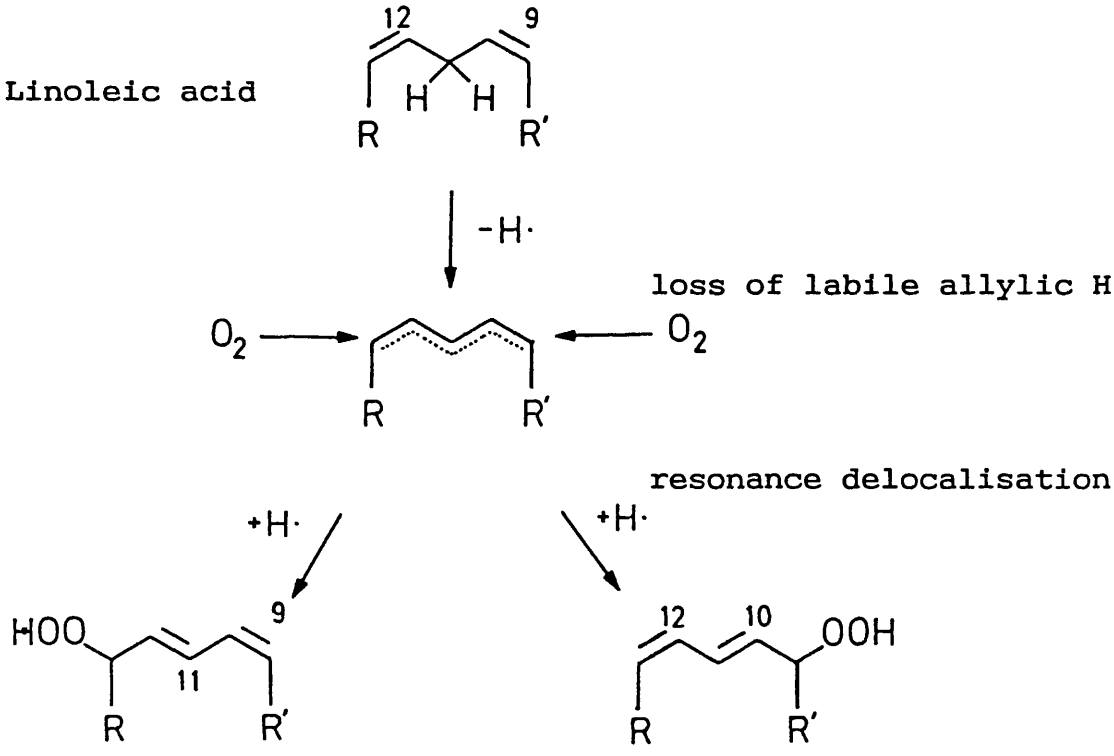
R: Linoleic/Linolenic acid chains

*: Propagation steps will cycle many times

Both acids have labile allylic hydrogens susceptible to the initiation of autoxidation as outlined in Figs.F.9 and F.10. It is probable that these mechanisms occurred in all three rhubarb samples, perhaps catalysed by transition metal ions (present in all food-stuffs[50]), exposure to light and heating. The formation of peroxyradicals was encouraged by the intimate contact of oxygen and the unsaturated acids during homogenisation.

Fig.F.9

The formation of linoleic acid hydroperoxide.[88]

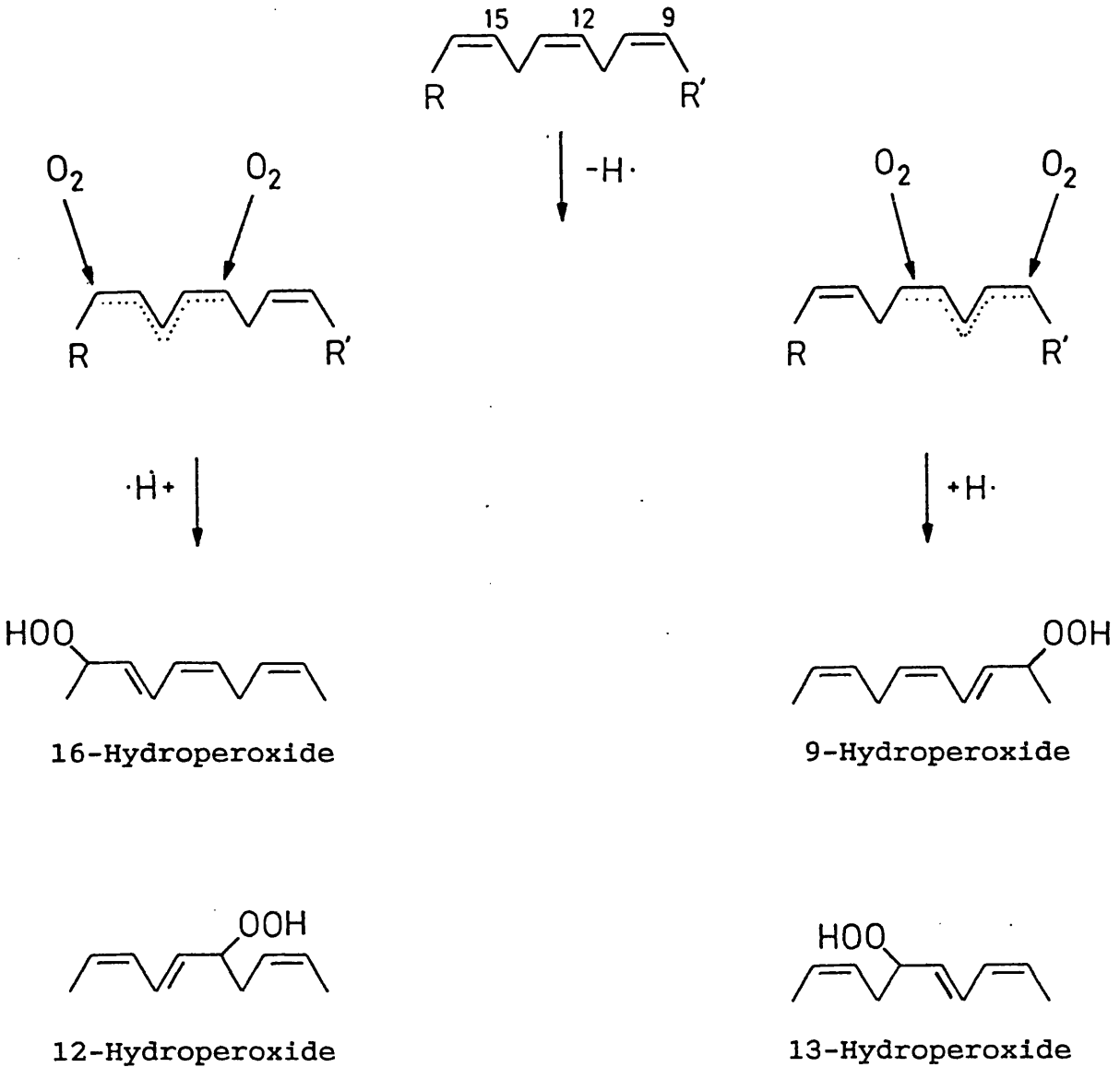


13-Hydroperoxide 9-Hydroperoxide

key R : non acid chain R' : chain containing acid group

Fig.F.10

The formation of linolenic acid hydroperoxide[88]



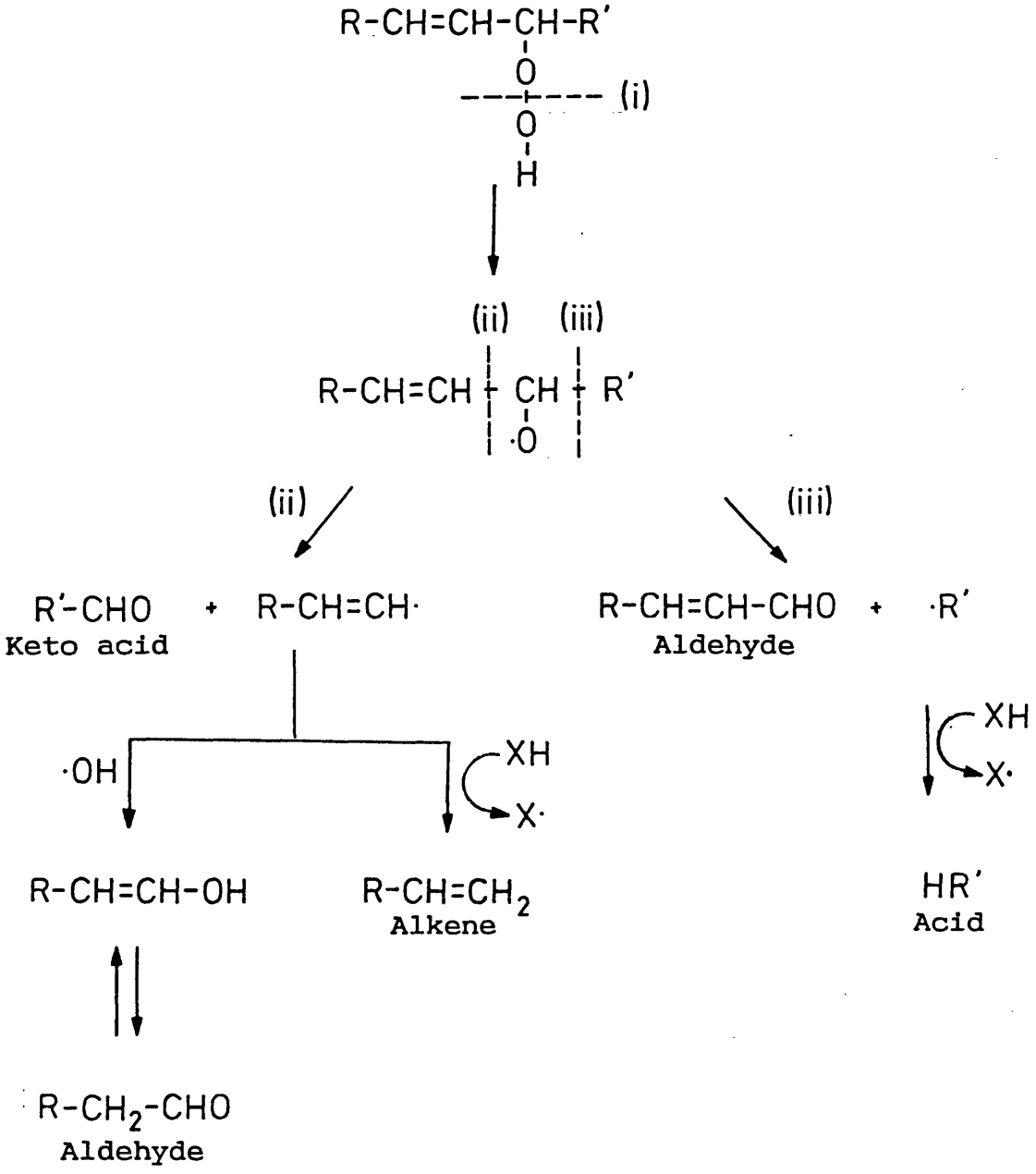
key

R : non-acid chain

R' : chain containing acid group

Fig.F.11

General mechanism for hydroperoxide decomposition.



key R : non-acid chain

R' : chain containing acid group

X : may be another unsaturated acid

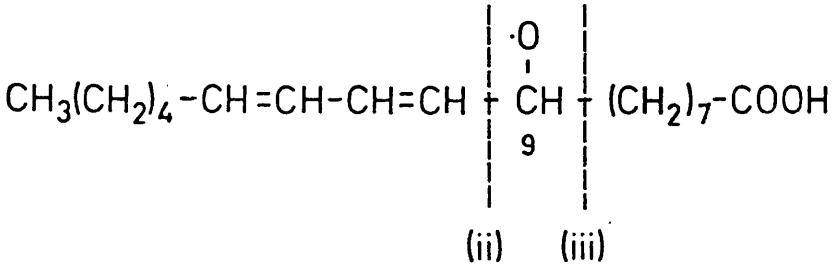
b) Breakdown of linoleic acid hydroperoxides

A general mechanism for hydroperoxide decomposition is shown in Fig.F.11 while Fig.F.12 details the specifics of linoleate hydroperoxide breakdown.

Fig.F.12

Linoleate hydroperoxide decomposition[88]

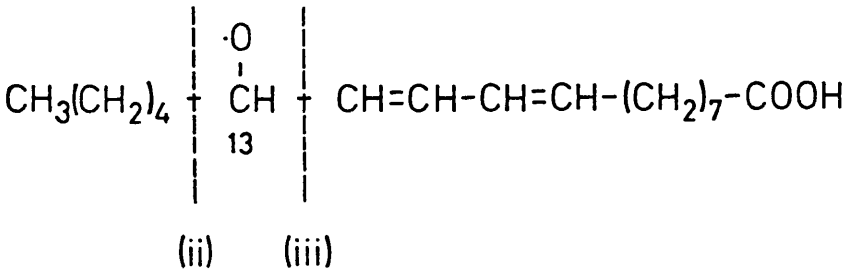
A) 9-Hydroperoxide



(ii) 3-Nonenal + 9-Oxononanoic acid

(iii) 2,4-Decadienal + Octanoic acid

B) 13-Hydroperoxide



(ii) Pentane + Pentanol + 13-Oxo-9,11-tridecadieneoic acid

(iii) Hexanal + 12-Oxo-9-dodecanoic acid

Only the 9- and 13- forms are outlined as the unconjugated 10- and 12- hydroperoxides are not normally produced during autoxidation. Further, analysis by several workers[50][89][90] has suggested that the oxidation of linoleic acid produces essentially those chemicals expected from the breakdown of the 9- and 13-hydroperoxides. Table VI.6 compares these products with those identified in the rhubarb extracts. It is evident that researchers have not, as yet, detected the alkenes predicted in Fig.F.11. In addition, the keto acids have also rarely been found, perhaps due to their instability and poor volatility under normal G.C. conditions.

Table VI.6 highlights the different volatiles produced from linoleic acid under various conditions. Elevated oxygen levels appear to favour the formation of C_8 volatiles such as 1-octen-3-one, 1-octen-3-ol, 3-octen-2-one and *cis*-3-octenal[90], while higher temperatures seem to promote various lower molecular weight volatiles. Further it is clear that linoleic acid produces more autoxidation products than those predicted in Figs F.11 and F.12. This is probably the result of the secondary reactions outlined later in c) d) and e). Few volatiles were identified in the cold dichloromethane extract of rhubarb suggesting that oxidation, be it chemical or enzymatic, had barely occurred. In contrast, the other rhubarb samples contained volatiles closely matching those produced by linoleic acid at elevated temperatures[89].

Table VI.6 Comparison of autoxidation products of linoleic acid and those found in rhubarb extracts

AUTOXIDATION PRODUCT	LINOLEIC ACID[90]	LINOLEIC ACID[89]	RHUBARB EXTRACT		
			1	2	3
Ethyl acetate	-	+	+	+	+
Pentanal	+	+	-	+	-
Hexanal	+	+	trc	+	trc
2-Heptanone	-	+	-	+	+
Heptanal	-	+	-	+	-
Pentanol	-	+	-	+	-
1-Octen-3-one	+	-	+	+	+
trans-2-Heptenal	+	+	+	+	+
1-Octen-3-ol	+	-	+	+	+
3-Octen-2-one	+	-	-	-	-
cis-3-Octenal	+	-	-	-	-
trans-2-Octenal	+	+	-	+	+
2-Nonenal	+	+	-	+	+
d-Hexalactone	-	+	-	-	-
2,4-Nonadienal isomers	+	-	-	-	-
cis,trans-2,4-Decadienal	+	+	-	-	+
trans,trans-2,4-Decadienal	+	+	-	-	+

Key

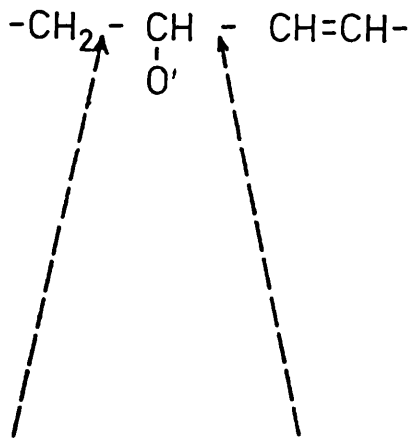
Linoleic acid[90]: heated at 24°C under oxygen

Linoleic acid[89]: heated at 250°C under air

1: Cold dichloromethane extract
2: Distilled sample
3: Preheated sample

Comparing Columns 2 and 3 in Table VI.6 reveals that while pentanol and hexanal were mostly identified in the distilled rhubarb, the decadienals were exclusive to the more severely heated, precooked rhubarb. This agrees with the results of several workers[91][92] including Henderson *et al.*[89] who noted that from 70°C to 180°C the total 2,4 decadienal level increased by 23%.

To explain this it was initially suggested that the cleavage position of linoleate hydroperoxides was temperature related[94]:-



High temperature cleavage Low temperature cleavage

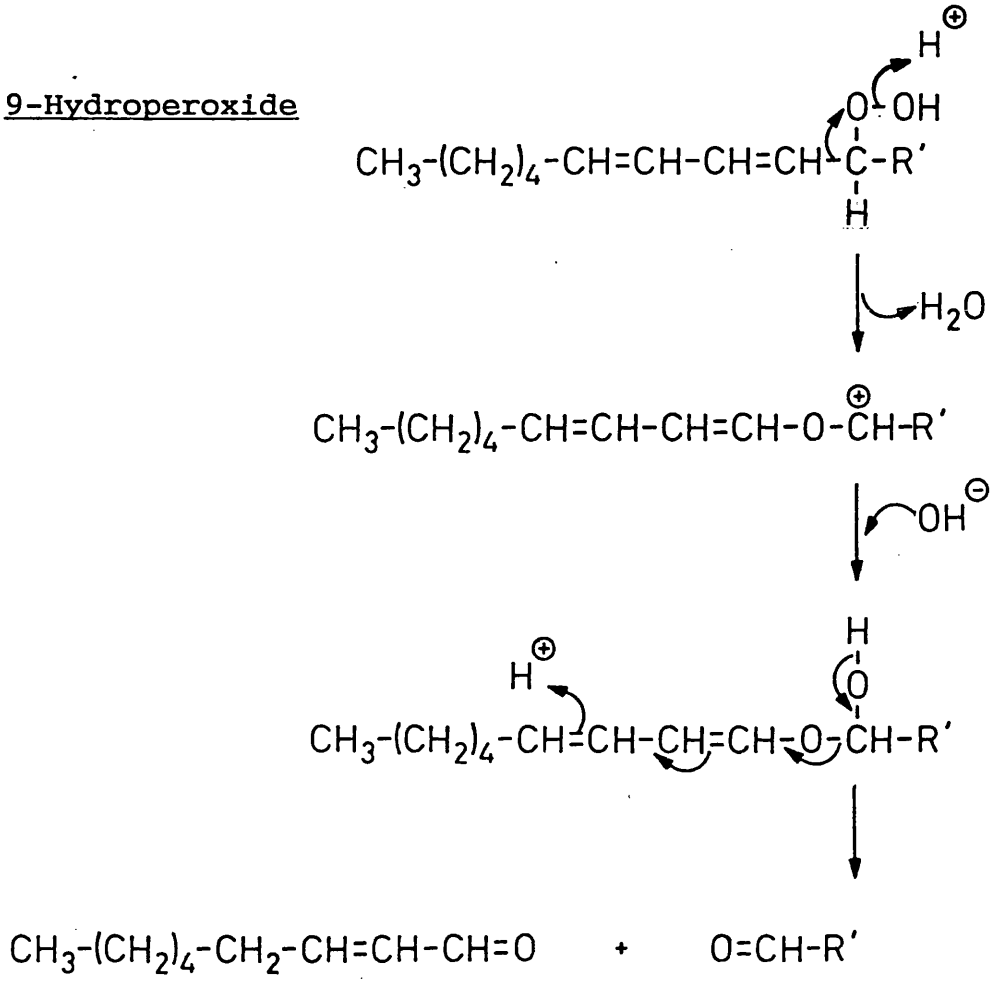
Transferring this theory to the postulated decompositions in Fig.F.12, low temperature should favour 3-nonenal and hexanal while high temperatures should favour 2,4-decadienal, pentane and pentanol. Ohloff[93] challenged this hypothesis by observing that the conjugation and hence the

resonance stability of 2,4-decadienal rendered it the most easily formed product, irrespective of temperature. It was left to Grosch *et al.* to ultimately explain the loss of 2,4-decadienal at lower temperatures by showing that the dienal itself could be oxidatively broken down by dissolved oxygen[97] [for details see: d) Secondary autoxidation of alkanals, alkenals and alkadienals]. At low temperatures oxygen remained in solution and could therefore oxidise the 2,4-decadienal, whereas prolonged high temperatures, such as those of preheated rhubarb, excluded oxygen and allowed the dienal to accumulate.

The ratio of the *trans,trans* and *trans,cis* isomers of 2,4-decadienal is also apparently affected by temperature. Ullrich and Grosch[90] and Henderson *et al.*[89] found ratios of 60:40 at 25°C; 74:26 at 70°C and 79:21 at 180°C for the *trans,trans* and *trans,cis* isomers respectively. When beef was heated to even higher temperatures the ratio was found to increase yet further[95]. Finally, Porter[96] proposed that high temperatures increased the concentration of hydroperoxides with a *trans,trans* diene system rather than a *trans,cis* one. The chromatogram of precooked rhubarb shows that, in agreement with Porter, the *trans,cis* isomer was present at trace levels only compared to the *trans,trans* one. Other workers have also suggested that the *trans,cis* configuration is formed preferentially when linoleic/linolenic acids are oxidised by enzymes and therefore, as is seen, would tend to be at a maximum at

Fig.F.13

The proton mediated autoxidation of linoleic acid.[97]



2-Nonenal

key

R' : Chain containing acid group

Fig.F.13 shows how protons can also mediate the autoxidation of 9-hydroperoxide. (13-hydroperoxides breakdown to give hexanal - an equivalent product to non proton autoxidation). It is not clear how significant proton mediated autoxidation is, but because of the highly

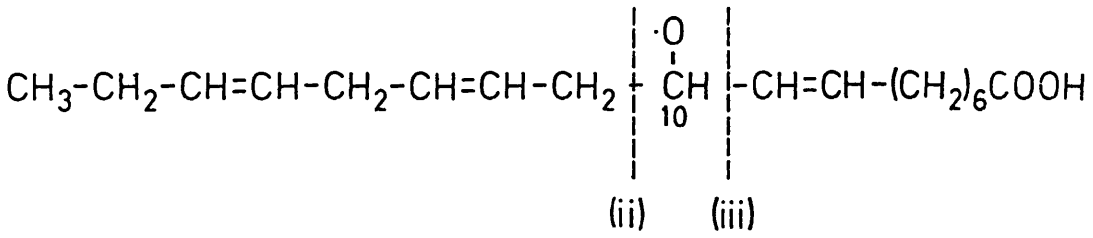
proton autoxidation). It is not clear how significant proton mediated autoxidation is, but because of the highly acidic nature of rhubarb stalk this mechanism may be favoured and thus influence the flavour of rhubarb. This method of breakdown may therefore explain the presence of 2-nonenal in heated rhubarb as well as in linoleic acid model systems (in the latter the acid itself functions as a proton source).

c) Breakdown of linolenic acid hydroperoxides

Frankel's postulated decomposition of linolenic acid is much more complex than that for linoleic acid. The decomposition of three types of hydroperoxides is shown in Fig.F.14. The actual autoxidation products from linolenate are shown in Table VI.7 along with relevant rhubarb volatiles.

Fig.F.14 Linolenate hydroperoxide decomposition[88][98]

A) 10-Hydroperoxide

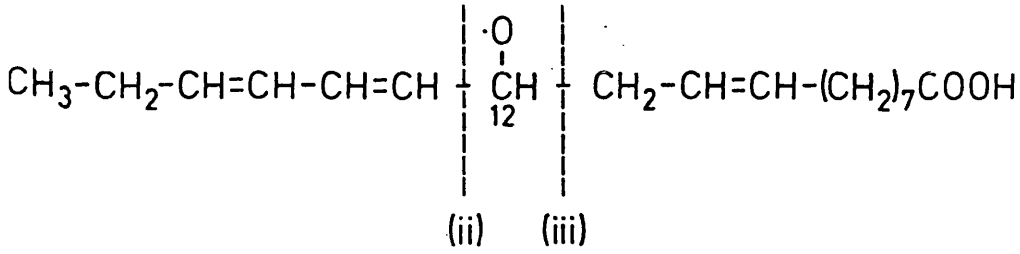


(ii) 2,5-Octadiene + 2,5-Octadienol + 10-Oxo-8-

decenoic acid

(iii) 3,6-Nonadienal + 9-Oxononanoic acid

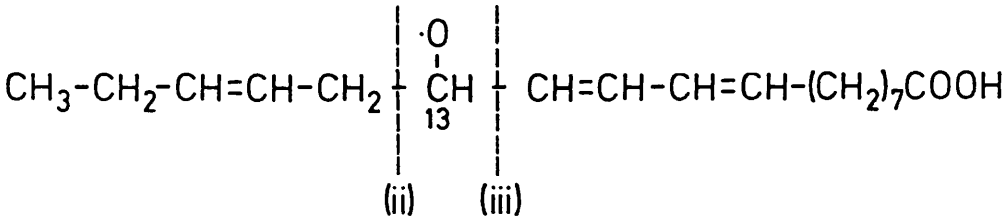
B) 12-Hydroperoxide



(ii) 3-Hexenal + 12-Oxo-9-dodecenoic acid

(iii) 2,4-Heptadienal + 9-Undecenoic acid

C) 13-Hydroperoxide



(ii) 2-Pentene + 2-Pentenol + 13-Oxo-9,11-tridecadienoic acid

(iii) 3-Hexenal + 12-Oxo-9-dodecenoic acid

The actual autoxidation products from linolenic acid are shown in Table VI.7

Table VI.7

Comparison of autoxidation products of linolenic acid and those found in rhubarb extracts[98]

AUTOXIDATION PRODUCT		RHUBARB EXTRACT		
	Mle	1	2	3
1-Penten-3-one	+	-	-	-
trans-2-Pentenal	+	-	-	+
cis-3-Hexenal	+	+	-	+
trans-2-Hexenal	+	-	+	+
1,5-Octadien-3-one	+	-	-	-
2,4-Heptadienal isomer	+	-	-	-
trans,trans-2,4-Heptadienal	+	+	-	+
trans,cis-3,5-Octadien-2-one	+	-	-	-
3,5-Octadien-2-one isomer	+	-	-	-
trans,cis-2,6-Nonadienal	+	-	-	-
Pentenyl furan	+	-	-	-

key

Mle : Methyl linolenate solution

1 : Cold dichloromethane extract

2 : Distilled sample

3 : Preheated sample

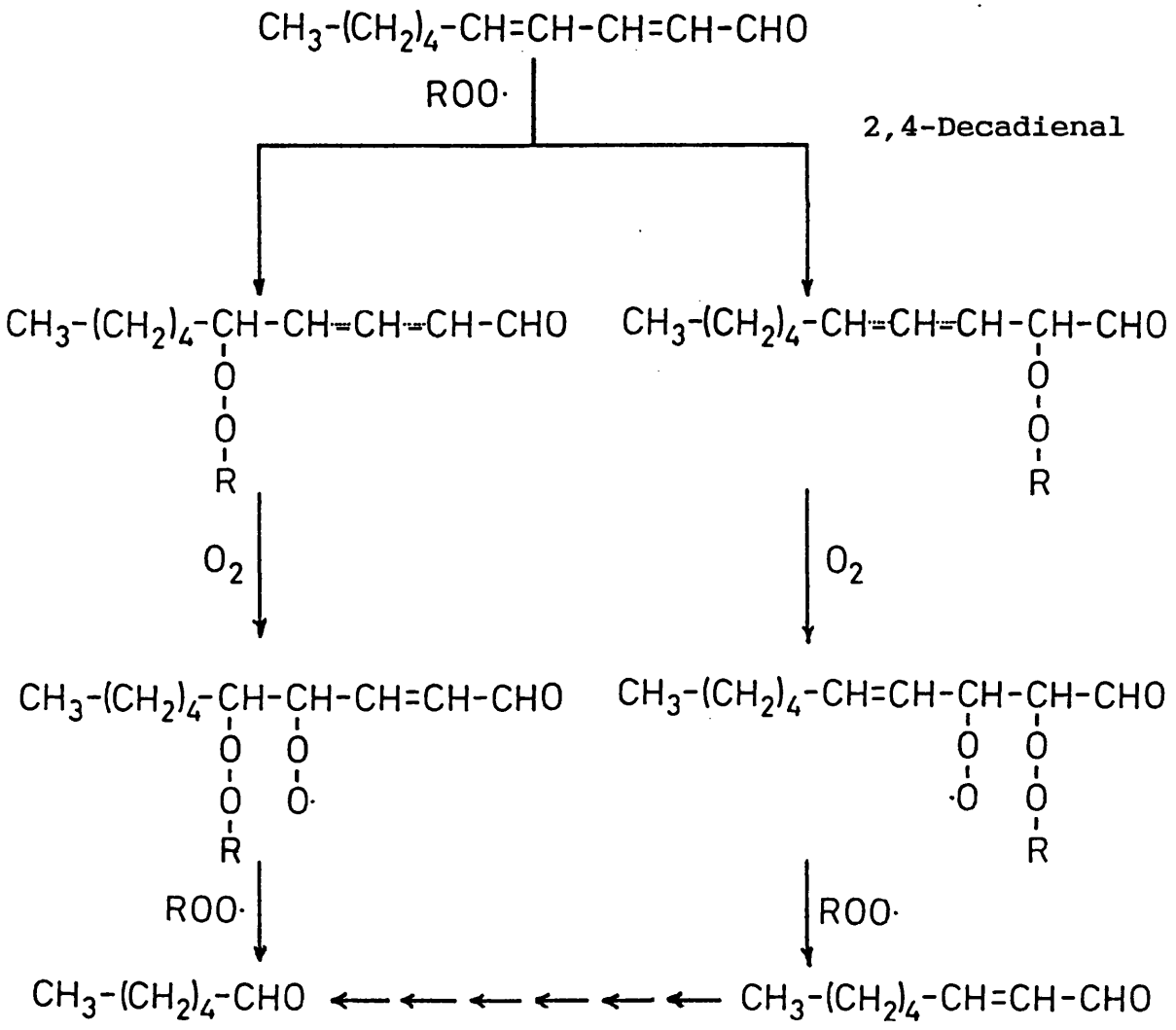
As with linoleic acid, it is probable that the breakdown products of linolenic acid in rhubarb can react and degrade further. These secondary processes are outlined in d) Secondary autoxidation of alkanals, alkenals and alkadienals and e) Retro-aldol related degradation of alkenals and alkadienals.

The presence of 2,4-heptadienal is often cited as evidence of linolenate autoxidation[50], however, the occurrence of this dienal in the cold dichloromethane extract indicates that it may also have been a component of intact stalks. This fact, coupled with the general absence of many of the other autoxidation volatiles, suggests that linolenic acid breakdown is of only minor importance in rhubarb.

d) Secondary autoxidation of alkanals, alkenals and alkadienals

Although the primary autoxidations outlined above can explain the presence of many of the constituents of rhubarb, as has already been said, secondary reactions can greatly increase the number of volatiles produced. One such secondary autoxidation is characterised in Fig.F.15.

Fig.F.15 The autoxidation of 2,4-decadienal[97]



The mechanism shown in Fig.F.15 is supported by model experiments in which *trans,trans*-2,4-decadienal was converted to significant levels of hexanal and 2-octenal[97][99]. These volatiles were also observed in the heated rhubarb extracts to which the 2-octenal provided a fatty/green character.

Other secondary oxidations that probably occurred in heated rhubarb involved the convertion of alkanals to their equivalent acids[50]. This process can occur quite readily and may have contributed to the increase in carboxylic acids outlined previously.

e) Retro-aldol related degradation of alkenals and alkadienals.

Sections a) - d) have explained how the autoxidation of linoleic and linolenic acids may lead to the formation of flavour volatiles in rhubarb. However, in an aqueous environment, those volatiles with a 2-alkenal structure are also able to undergo a further non-oxidative breakdown, namely alpha/beta double bond hydration coupled to retro-aldol condensation. This process promotes stale flavours in fried foods[100] and the loss of 2,4-decadienal in aqueous slurries of meat held at elevated temperatures[101]. Fig.F.16 details the reaction pathways for 2-alkenals and 2,4-alkadienals[100][102][103] hypothesised from model heating trials under O_2 and N_2. Breakdown was found to be independent of oxygen levels and experiments at pH 2.8 and 7.5 showed that the products were similar at each pH.

Fig.F.16 Alpha/beta double bond hydration and retro-aldol condensation in A) 2-alkenals B) 2,4-alkadienals

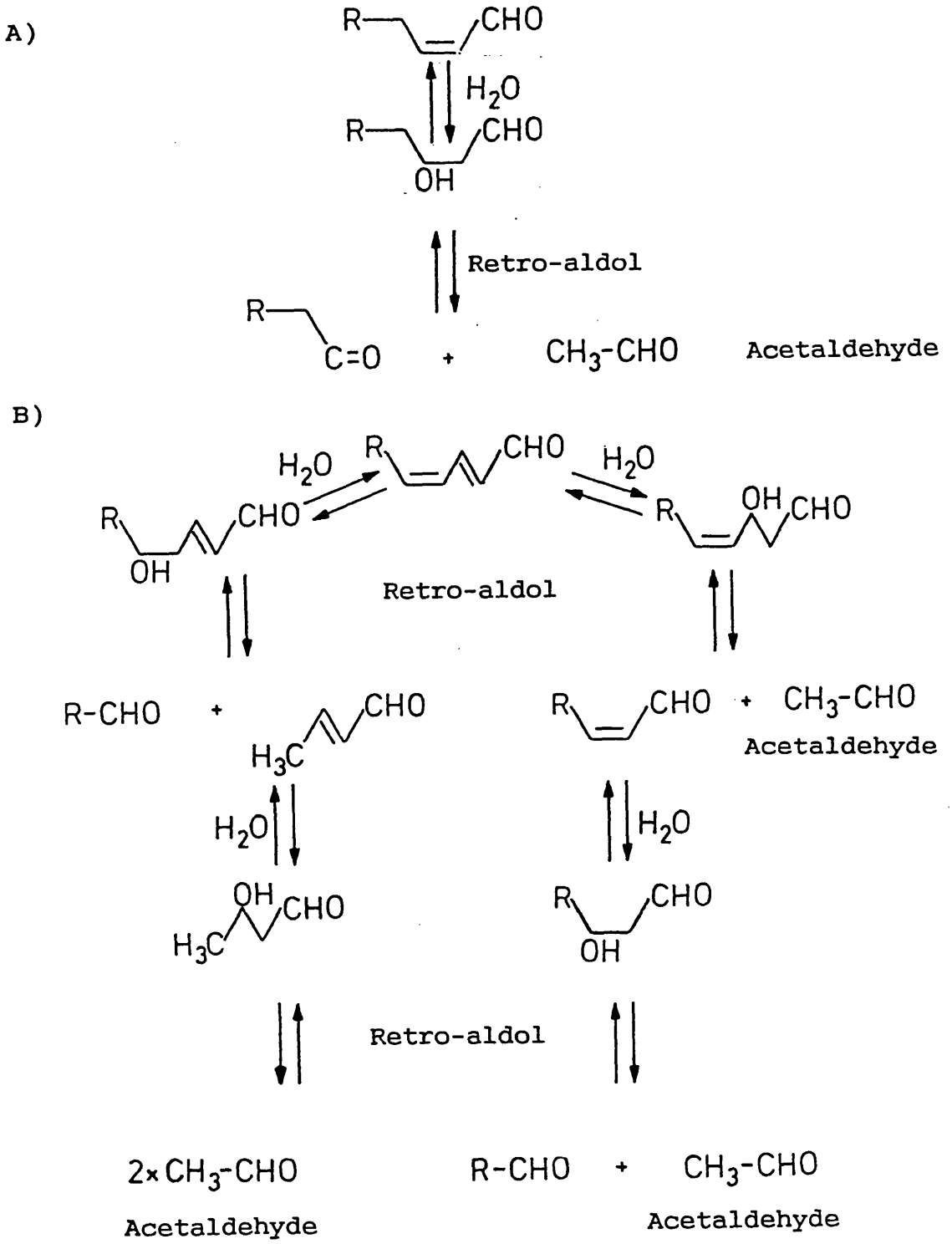


Table VI.8 compares these products with those found in rhubarb and shows that many retro-aldol condensation products had formed after heating. Oxygen dissolved in the homogenised rhubarb stalk would have been driven into the head-space during heating, and this, coupled with the pH of 3.6, could have been compatible with retro-aldol condensation occurring in the homogenate.

Table VI.8
Alpha/beta double bond hydration/retro-aldol condensation products and presence in rhubarb extracts

	*	RHUBARB EXTRACT		
PRECURSOR	RETRO ALDOL CONDENSATION PRODUCT	1	2	3
trans-2-Decenal		-	+	+
	Octanal	+	-	+
trans-2-Nonenal		-	+	+
	Heptanal	-	+	+
trans-2-Heptenal		+	+	+
	Pentanal	-	+	-
trans,trans-2,4-Decadienal		-	-	+
	trans-2-Octenal	-	+	+
	Hexanal	trc	+	trc
trans,cis-2,4-Heptadienal		-	-	-
	trans-2-Pentenal	-	-	+

key

* lower M.W. products such as acetaldehyde were not analysed

1: Cold dichloromethane extract

2: Distilled sample

3: Preheated sample

To conclude, it is obvious that a high degree of oxidative linoleic/linolenic acid breakdown occurs in rhubarb. However, the mechanism of secondary decomposition of unsaturated aldehydes is much more obscure. Examination of Tables VI.6, VI.7 and VI.8 demonstrates that many rhubarb volatiles could be formed by either autoxidation or retro-aldol condensation. Indeed, both processes may be occurring simultaneously.

Flavour analysis has shown that many of the unsaturated compounds discussed in this section have low threshold values and therefore have a potential effect on the flavour of rhubarb. In the commercial exploitation of rhubarb as a flavouring, the consideration of temperature, homogenisation and oxygen conditions during processing, might be useful in optimizing the levels of these flavour volatiles.

2) The dichloromethane extraction of canned rhubarb

A large percentage of rhubarb is eaten after being canned and as such it was considered important to identify components specifically resulting from canning. Comparing the chromatograms of the canned and other rhubarb extracts (Chapter 4: Figs.D.4, D.1, D.2 and D.3) reveals that although they share many similarities, the levels of early running compounds, such as alcohols, carbonyls, esters and terpenes were relatively lower in the canned sample. This may be a result of the blanching process (see Fig.F.17) which involved heating to 140°C in open vessels, and hence would have allowed some evaporation of these more volatile compounds.

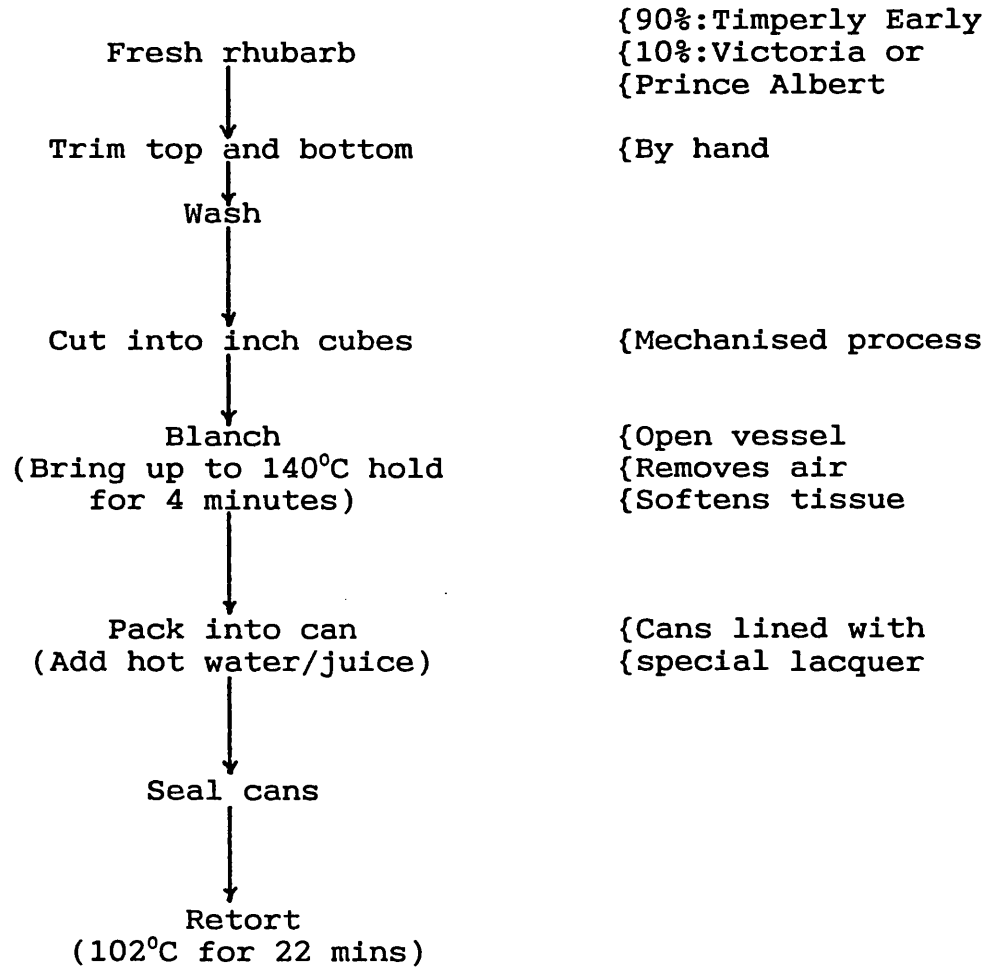
The aroma description of the canned extract was acidic, sulphurous, vegetal, metallic, cooked, non-green and generally weaker than the other heated samples.

Fig.F.17 outlines the commercial process involved in rhubarb canning. It is notable that 90% of the cans available at supermarkets contained Timperly Early, the same variety employed in earlier analyses. Although it was not possible to positively confirm whether the cans investigated contained Timperly Early, trials in this laboratory (unpublished data) have shown that rhubarb varieties appear to differ merely in the quantity, rather than the type, of flavour volatiles. It was safe

therefore, to directly compare the canned extract with the other samples, and to say that the presence of components not previously identified was a function of the processing and not of any varietal differences. Such components included various ketones, saturated alkanes, pyridine, isomers of methylnaphthalene, plasticisers and quinoline; these are discussed below.

Fig.F.17

The commercial canning of rhubarb



Ketones

Several ketones appear in the canned rhubarb sample that were not identified in previous samples. However, investigation of other foods[24] has not revealed a particular correlation between the appearance or presence of these ketones and the effects of heating. Table VI.9 details the ketones, gives a flavour/aroma description and identifies other heated foods in which they have been found.

Table VI.9

The ketones specific to canned rhubarb, their flavour description[104] and other cooked sources

	Description	Other cooked sources
2-Butanone	Ethereal, lifting	Many
3-Methyl-2-butanone	Ethereal, vinous	Potato, Peanut, Bean
3-Pentanone	Acetone like, warm, pleasant, diffuse	Cabbage, Butter

2-Methyl-3-pentanone	Ethereal, lifting, warm, citrus/fruit	Onion, Peanut
Mesityl-oxide	gassy, green, pungent, honey	Potato, Peanut, Almond
3-Hydroxy-2-butanone	Butter	Many

In the flavour industry 2-butanone is frequently added to flavours such as apricot and peach and seems to be consistent with a processed, heated character. This chemical may typically lend this flavour nuance to canned rhubarb.

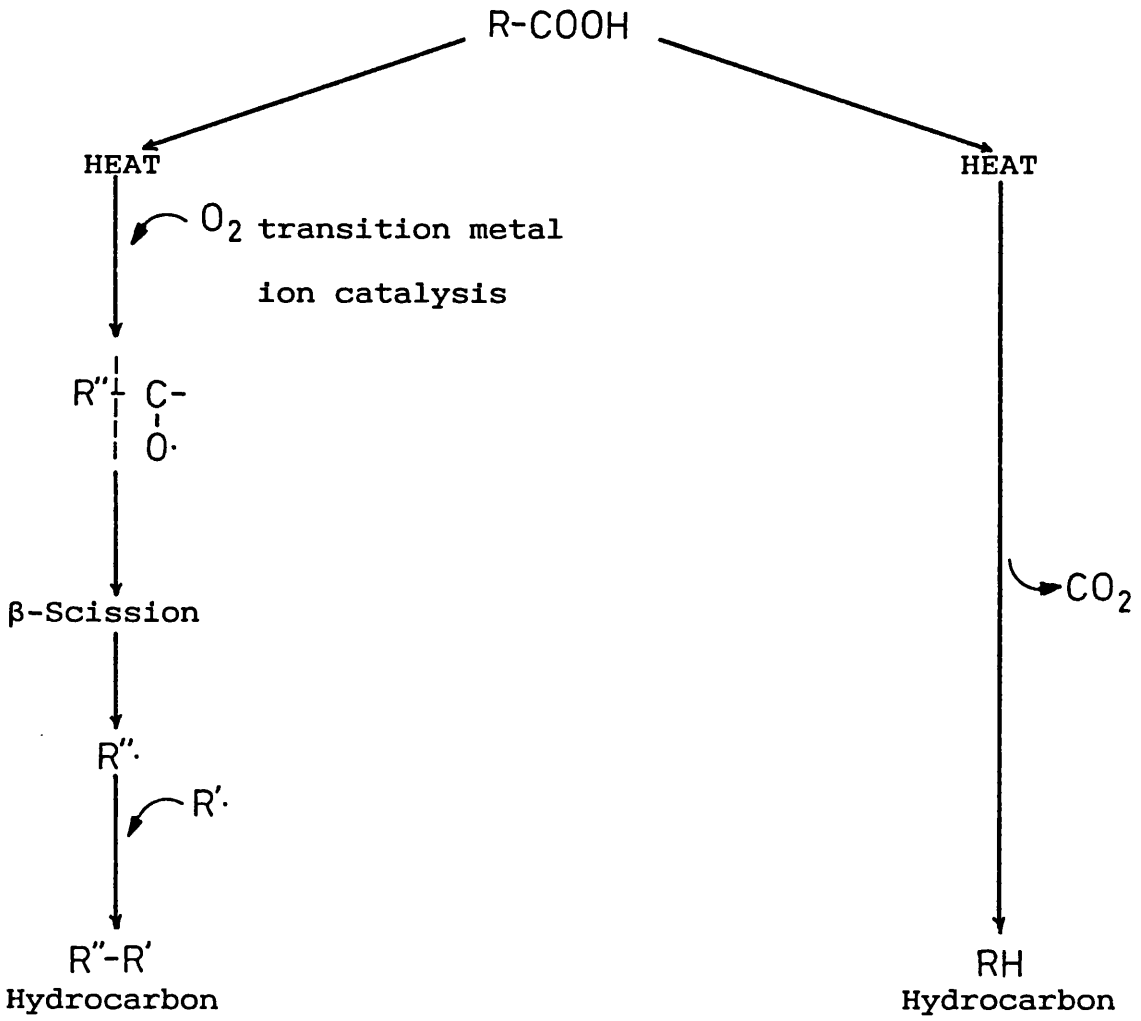
3-Hydroxy-2-butanone (acetoin) is a component of many heated foods and has been confirmed, along with furfural, as a product of carbohydrate caramellisation[41]. Acetoin can also arise from the early stages of the Maillard reaction and, as rhubarb is known to contain 0.14%m/m total nitrogen and 0.8% m/m total sugar[15], this mode of formation is quite likely during canning. It is interesting to note that other sugar thermolysis products, such as 5-hydroxymethylfurfural and methyl 3-furoate, were not detected, and hence the extract more closely resembled the distilled rather than the preheated rhubarb.

Alkanes

It is widely known that a significant number of hydrocarbons develop when lipids are thermally degraded. The two mechanisms which have been proposed for the formation of these hydrocarbons, from both saturated and unsaturated fatty acids, are outlined in Fig.F.18.

Fig.F.18

The formation of Hydrocarbons *via* the thermal degradation of Fatty acids



These processes are well documented for high fat systems[24] such as heated meats, and might well play a significant role in the formation of hydrocarbons in canned rhubarb. While, in comparison to meat, there is a relatively low concentration of lipids in rhubarb, the degradation shown in Fig.F.18 may be exacerbated by the higher levels of metal ions encountered during the canning process.

Aliphatic hydrocarbons have relatively high odour thresholds and are therefore unlikely to have a major effect on the flavour of canned rhubarb.

Pyridine and methylnaphthalenes

In this study, pyridine was unique to the canned rhubarb extract. During the analysis of the flavour components of rice, Yajima *et al.* observed that pyridine was only detectable after cooking and was tentatively assigned as a product of amino acid breakdown[53][61][105]. If this were the case in rhubarb it is difficult to explain why it was absent in other heated samples. Cooked beetroot has also been found to contain high levels of pyridine but this was related to the thermolysis of betalain pigments[106], as yet not found in rhubarb. However, the most likely source of pyridine in canned rhubarb was *via* contamination with rhubarb root (work in this laboratory has shown that pyridine is a significant component of edible rhubarb root). Similarly, the two isomers of methylnaphthalene

detected in canned rhubarb may also be attributable to the presence of several substituted naphthalenes observed in edible and Chinese rhubarb root[9]. Alternatively, methylnaphthalenes can form *via* the thermal breakdown of phenylalanine[41] and indeed have been observed in heated products such as coffee and black tea[24].

Contaminants/artifacts

Tributyl phosphate and the three trimethyl-1,3-pentanediol esters are known plasticisers and therefore could be contaminants. Tributyl phosphate was peculiar to the canned sample and may have been leached from the lacquer[58] on the interior of the cans. B.H.T. is an antioxidant which, as it was not declared as an additive on the cans, may have been a contaminant originating from the laboratory.

Quinoline

Quinoline is described as a nature identical chemical in the FEMA GRAS listing[107] and has a character similar to benzaldehyde and anise. It has been found in several products after heating such as rice, tea and tobacco[105][108][109][110], as well as in the canned rhubarb

extract. The quinoline structure is the basis of several alkaloids in the plant kingdom (eg quinine) and it may be speculated that quinoline is formed *via* the breakdown of a similar alkaloid in rhubarb.

3) **The liquid/liquid dichloromethane extraction of fresh rhubarb and quantitation of volatiles**

Table IV.5 (Chapter 4) charts the fluctuation in concentration of thirty-eight rhubarb components throughout the growing season of 1991. Observing the total peak area for each sample (excluding standards), it can be seen that Sample 1 (27th May) contained the maximum quantity of volatiles and that over the next 6 weeks levels declined by 50%. The final sample harvested on 18th July did, however, show an increase in volatiles back to 80% of the Sample 1 value. This latter effect may be linked to the poor cropping of the rhubarb plants by mid-July, wherein the rhubarb volatiles are perhaps concentrated into the fewer stalks produced.

Clearly, taken as an entity, rhubarb harvested early in the season has the greatest flavour, and hence, commercial value. However, for a rhubarb grower the yield of stalks/plant is also a significant factor. Indeed, if in late June the stalks are found to contain 69% of the flavour potential of earlier, but double the crop is produced, then obviously this later harvest is more profitable. In contrast, the extra cost of processing these less flavoured stalks may mean that the early crop is the preferred choice for the natural flavour producer. Ascertaining the maximum flavour achievable for the minimum cost would necessitate large scale trials observing stalk

yield/acre when harvesting at different times during the season. Further, total quantitation and costing for the commercial production of a volatile from these harvestings would also be required.

Observing the volatile fluctuations in Table IV.5 reveals some interesting patterns. The level of terpenes such as limonene and linalool showed a steady decline throughout the period of harvesting, a trend generally followed by most of the early running, volatile components. Later components appeared relatively unchanged throughout the seven weeks. Aromatics, apart from phenylacetaldehyde, did not decline and indeed the salicylaldehyde concentration was three times higher in later harvestings than earlier.

The values for acids were variable, e.g. heptanoic and decanoic acids decreased over time, octanoic acid increased, while the others fluctuated throughout. Carboxylic acids are notoriously difficult to quantify by G.C. and so the figures here may well be of limited value.

The thirty-eight components were quantified using a DB-WAX column and this, combined with their earlier elucidation on DB-5, acted as a confirmation of their presence in rhubarb. Frambinone and γ-butyrolactone were identified for the first time, perhaps suggesting that they had previously been lost under other peaks on the DB-5 column.

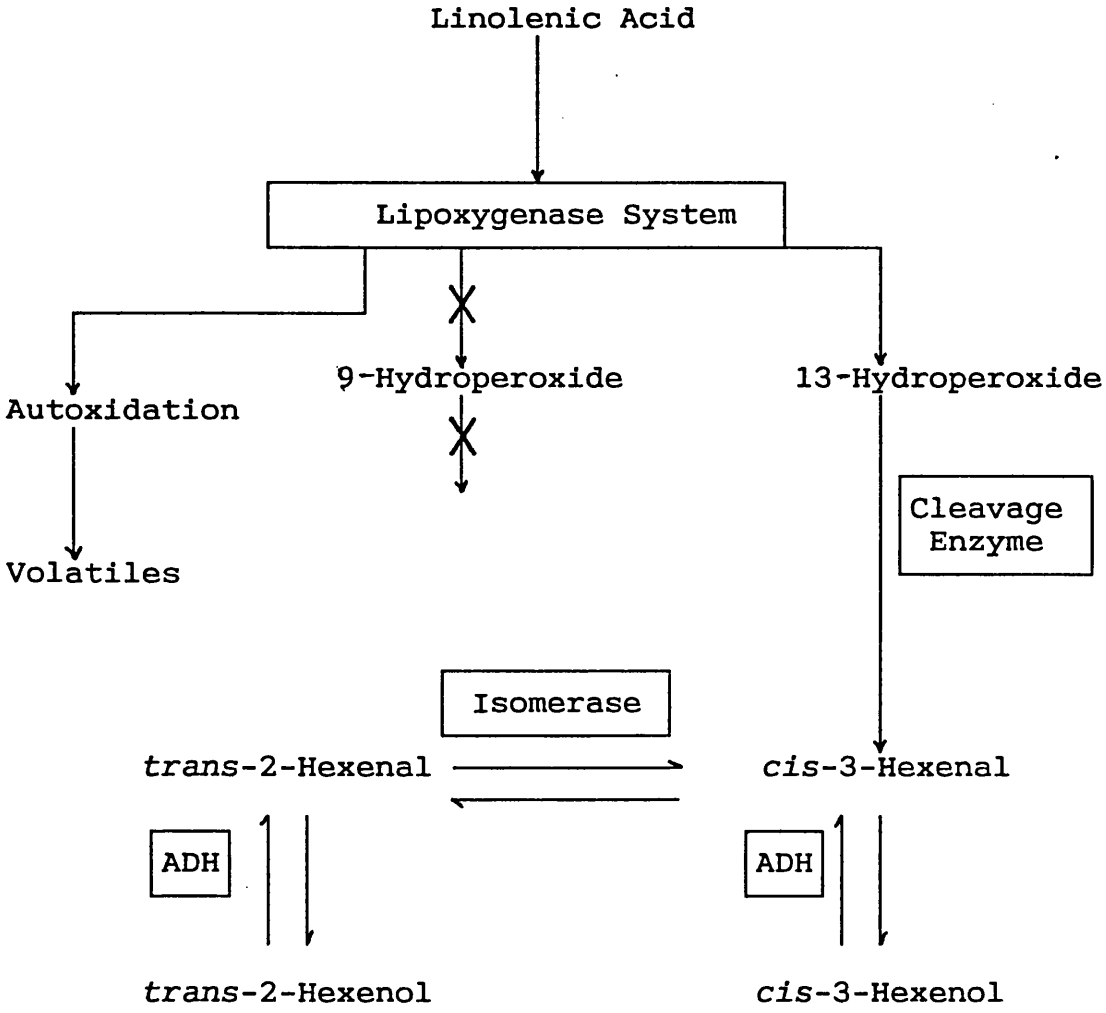
4) The volatiles derived from the enzymatic hydrolysis of linoleic/linolenic acids in rhubarb

The processing of rhubarb for flavour production is likely to involve homogenisation and aeration, conditions ideally suited to linoleic and linolenic acid. The autoxidation of these precursors is covered in some detail earlier in this chapter. Here however, the enzymatic hydrolysis of the unsaturated fatty acids and the subsequent effects on rhubarb flavour, are discussed.

A study was carried out to investigate the formation of volatiles over time and at different pH. This discussion firstly summarises what is known of the enzymatic breakdown of linoleic/linolenic acids and then relates this to the results found in rhubarb (Chapter 4: Table IV.6, Fig.D.6 and Table IV.7, Fig.D.7). Some conclusions are also drawn on methods by which these enzymatically derived volatiles might be maximised during rhubarb processing.

A general overview of the breakdown mechanism, proposed for rhubarb, is shown in Fig.F.19. The enzymes involved in the oxidation of linolenic acid, from lipoxygenase to alcohol dehydrogenase, are detailed. The point where autoxidation may link in with this enzymatic pathway is also indicated.

Fig.F.19
Schematic showing the possible mechanisms for the formation of volatiles from Linolenic acid in rhubarb



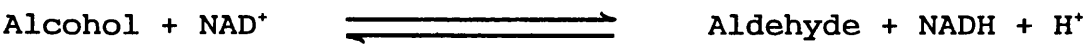
Lipoxygenases require activation before they are able to catalyse the breakdown of unsaturated acids. The attachment of linole(n)ic acid hydroperoxides to specific sites on the enzyme induces conformational changes in its catalytic structure and initiates substrate binding. This activation by the enzyme's product is an example of positive feedback and encourages maximum hydroperoxide formation[111]. Linole(n)ic acid then binds to the active site, along with an oxygen molecule, and hydroperoxide is synthesised and released[112]. Lipoxygenases can show specificity for the position of peroxidation on the unsaturated fatty acid, e.g. cucumber enzymes produce 75% peroxidation at Carbon 9 and 25% at Carbon 13 of linole(n)ic acid[113], whereas the percentages in apple are 15% and 85% respectively[114]. In many plants there exist enzyme cleavage systems which can break down the hydroperoxides synthesised above. In this way linoleic and linolenic 9-hydroperoxides can be further metabolised to *cis*-3-nonenal and *cis,cis*-3,6-nonadienal respectively. In an analogous mechanism 13-hydroperoxides are converted to hexanal and *cis*-3-hexenal.

In practice a range of C_6 and C_9 volatiles is produced enzymatically from unsaturated acids. This is because of the further activity of isomerase enzymes and alcohol dehydrogenases (ADH) - see Fig.F.19. (*trans*)(*cis*)-2,3-Enal isomerase is an example of such an enzyme which, in couch grass[115], catalyses the almost complete conversion of *cis*-

hexenal to *trans*-2-hexenal. Fig.F.20 shows how ADH can interconvert aldehydes and alcohols such as those from fatty acid peroxidation.

Fig.F.20

Interconversion of Alcohols/Aldehydes *via* ADH

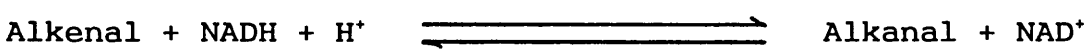


The relatively high NADH level and low pH of many plants tends to drive Fig.F.20 to the left, favouring alcohol formation[116].

Fig.F.21 shows how ADH is also understood to partially reduce an unsaturated aldehyde to a saturated aldehyde[117].

Fig.F.21

Interconversion of Alkenals/Alkanals *via* ADH



Enzyme activity can then, as described, lead to the production of a complex array of potent chemicals in broken plant tissue.

Discussion of rhubarb results in relation to enzyme activity:-

Fig.D.6 clearly demonstrates the involvement of enzymes in volatile formation during homogenisation. The results indicate that methanol was effective in inhibiting any enzymes involved in unsaturated-acid breakdown. In addition, the specific inhibition of lipoxygenase may also greatly reduce the level of hydroperoxides available for volatile formation *via* autoxidation (see earlier in this chapter for mechanisms).

As alkenals are in the majority over alkanals it may be surmised that linolenic acid is a more prolific substrate than linoleic acid. However, as a *caveat*, it is important to note that ADH can partially interconvert alkenals and alkanals (Fig.F.21) and therefore linoleic acid may also be a source of some of the 'alkene' volatiles detected.

The exclusivity of 13-hydroperoxide breakdown products suggests that i) rhubarb lipoxygenase only produces ^{13}-hydroperoxides (peanut shows a similar specificity[113]) or ii) the rhubarb hydroperoxide cleavage system, if present, is specific for 13-hydroperoxide (analogous to tomato[118]).

After 120 minutes, the level of aldehydes (calculated total 2154μg/kg) was much higher than alcohols (137μg/kg) in the homogenised rhubarb. This indicates that there was limited

ADH-catalysed conversion of the available C_6 aldehydes into alcohols.

Apples and grapes show a similar bias towards aldehydes whereas homogenised tomato appears to exhibit a high ADH activity[112]. The consistently low level of *cis*-3 volatiles relative to *trans*-2 volatiles suggests that rhubarb may contain a (*trans*),(*cis*)-2,3-enal isomerase, which is active from the moment of homogenisation.

Fig.D.6 indicates that the storage of homogenised fresh rhubarb for 60-120 minutes caused effects ranging from a 2.5-fold increase in *cis*-3-hexenal to a 15-fold increase in *trans*-2-hexenal. Clearly this storage would significantly increase the green, leafy character of rhubarb. In the commercial production of rhubarb flavour however, holding for periods longer than this might cause undesirable deterioration and could increase the danger of contamination.

Fig.D.7 reveals that the enzyme system forming *trans*-2-hexenal has a low pH optimum, and in rhubarb flavours it is clear that the level of *trans*-2-hexenal could be adjusted by altering the pH. Recently published information has shown that freezing and thawing can disrupt lipoxygenase activity[119] and as such may have affected the pH optima of the rhubarb enzyme system assessed here.

CHAPTER 7

DISCUSSION OF RESULTS

RHUBARB GLYCOSIDES

It can be seen from the results (Chapter 5:Fig.E.1 - E.5) that upwards of 64 components occurred in the aglycone fraction of rhubarb stalk, 113 in the aglycone fraction of rhubarb leaf and 10 in the aglycone fraction of rhubarb root. Of these compounds, 50, 68 and 6 respectively, were identified in each sample.

Some of the components were identified only tentatively because standard chemicals were unavailable, nevertheless each was subjected to mass spectrometry and this data was then used as uncorroborated evidence of a chemical's identity. The chromatographic traces from the DB-5 column show poor resolution and the peaks are broad, whereas early eluting compounds did not separate and tended to run with the solvent front. Better separation was obtained on Carbowax. This may be an effect of the column polarity rather than of the individual columns, indeed it is notable that other laboratories have generally employed Carbowax phases for analysis (approx. 70%).

The results show that in rhubarb plants the number of glycosides increases from the root, through the stalk and into the leaf. This distribution is mirrored in other plants including *Hyssopus officinalis*[120], ginger[121], blackberry [55], redcurrant[122] and sloe[123]. Generally it seems that norisoprenoid glycosides are prevalent in the leaves whereas shikimate-derived glycosides predominate in the roots.

These differences are discussed below in what is the first investigation into the volatile aglycones in rhubarb.

Fig.G.1 shows a schematic of the three categories of volatiles bound as glycosides in rhubarb. The alcohol category is subsectioned into four groups. Each category will be referred to in turn.

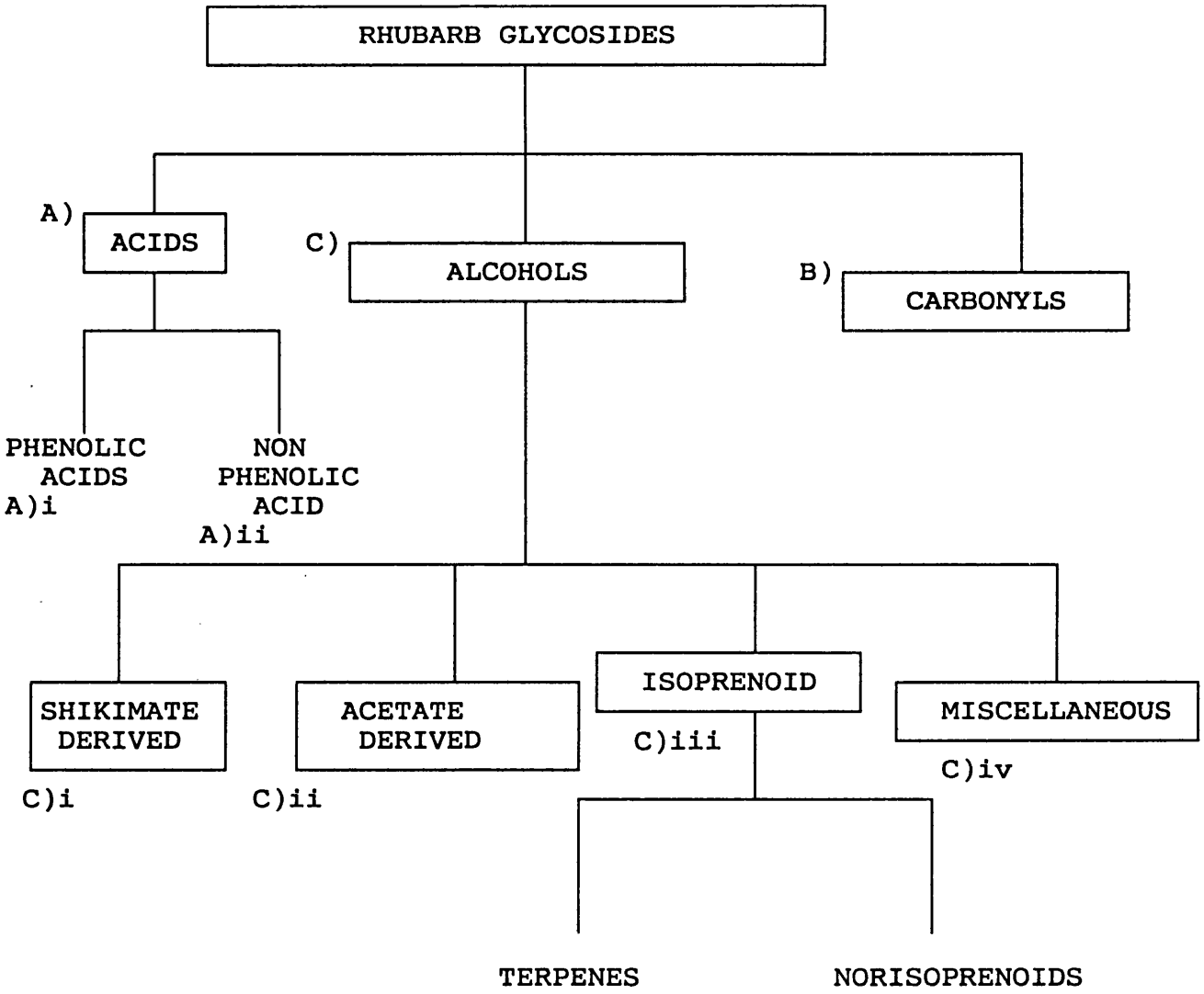
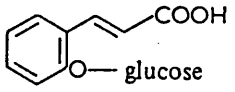


Fig.G.1. The categories of volatiles present as glycoside in rhubarb

FLAVOUR VOLATILE GLYCOSIDES

RHUBARB GLYCOSIDES: A) ACIDS

Although there is an absence of work which has specifically looked at the linkage between volatile organic acids and sugar moieties, they are most probably ester or ether bonds. Glucose ether formation is well documented in plant tissue, especially with coumaric, caffeic and ferulic acid.



Coumaryl glucoside

Rhubarb acid glycosides can be separated into two groups:- i) Phenolic acids and ii) Non-phenolic acids

i) Phenolic acids

The volatile phenolic acids; benzoic, phenylacetic and *trans*-cinnamic acid were identified as aglycones released from rhubarb glycosides. It is also likely that other less volatile phenolic acids (such as those mentioned above) are also present as glycosides in rhubarb. It has been proposed that these phenolic acid glycosides act as

intermediates in chlorogenic acid synthesis[26]. Alternatively, glycoside formation may act as a detoxification process whereby phenolics can be safely stored in living cells, without much metabolic turnover.

Benzoic acid aglycones have also been identified in grape[124], strawberry[125], apricot, peach, plum[126], raspberry[127], blackberry[55], lulo fruit[128], tomatoes[129], mango[130], papaya[131] and hog plum[132]. *trans*-Cinnamic acid has been found in strawberry[125], raspberry[127], blackberry[55], pineapple[133] and hog plum[132], while phenylacetic acid was detected in grape[124] and papaya[131].

ii) Non-phenolic acids

Straight-chained fatty acids, acetic, butyric, hexanoic, *cis*-3-hexenoic, *trans*-2-hexenoic, octanoic and dodecanoic acid and the branch-chained 2-methylbutyric acid were all found as aglycones in rhubarb stalk, leaf and/or root.

Various combinations of these non-phenolic acids have also been observed in ginger[121], strawberry[125] (where 2-methylbutyric acid was found to be present in the enantiomerically pure (*S*) form), apricot, peach, plum[126], raspberry[127], blackberry fruit and leaf[55], lulo fruit[128], tomato[129][134], pineapple[133], mango[130], papaya[131] and hog plum[132]. Only strawberry and raspberry were found to have as great a number of bound fatty acids as rhubarb. *cis*-3-

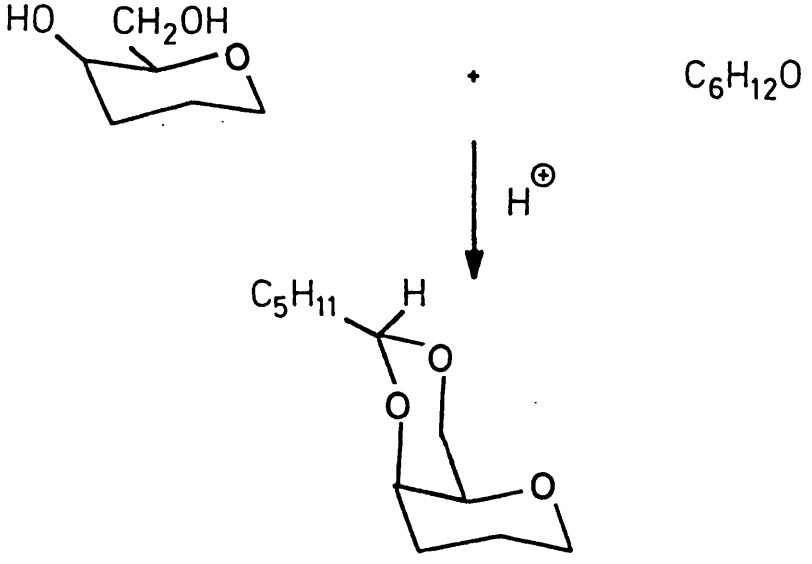
Hexenoic acid does not appear to have been identified in the bound form before while *trans*-2-hexenoic acid has only previously been found in strawberries.

RHUBARB GLYCOSIDES:B) CARBONYLS

Hydrolysis of these glycosides releases aldehydes or ketones as aglycones.

Occasionally aldehydes such as hexanal and *trans*-2-hexenal have been characterised as agylcones. Buttery *et al.*[129] suggested that these aldehydes are actually present unbound but form acetals with free sugars in acid environments (Fig.G.2).

Fig.G.2 Acetal formation with free sugars

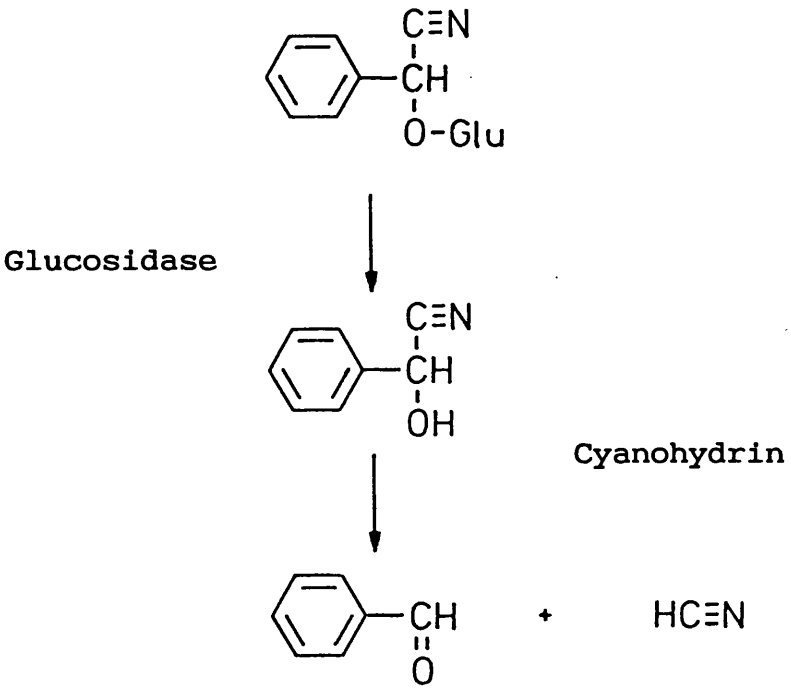


Cyclic Acetal

The identification of octanal as an aglycone in rhubarb may be explained in a similar manner.

Benzaldehyde is well documented as being present in plant tissue in the form of a cyanogenic glycoside.[26] An example is prunasin in peach (Fig.G.3).

Fig.G.3. The hydrolysis of prunasin



Benzaldehyde is released from the cyanogenic glycoside by the action of a glucosidase. Initially a cyanohydrin is formed which then undergoes non-enzymatic degradation to benzaldehyde and hydrogen cyanide. Similar processes are the most probable explanation for the identification of benzaldehyde in rhubarb leaf and root.

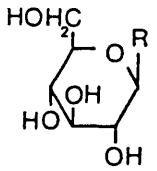
Bound benzaldehyde is also known to be present in grape[124], passion fruit[135], ginger[121], apricot, peach, plum[130] and sloe leaf.[123]

In general, aromatic carbonyl aglycones in plants are thought to originate from aromatic amino acids e.g. benzaldehyde from phenylalanine and 4-hydroxybenzaldehyde from tyrosine. The origins of anisaldehyde, another aglycone in rhubarb is less clear, indeed this is the first time it has been reported as a bound component in plants.

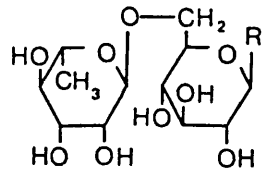
Although the sugar moieties of rhubarb glycosides have not been investigated here, such work has been pursued in many other plants. Fig.G.4 shows the four most common sugar moieties in grapes as determined by Voirin *et al.*[136], and which may be bound to many of the volatile rhubarb aglycones as well.

Fig.G.4

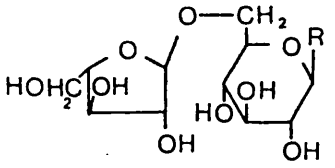
Common sugar moieties found present as glycosides in plants

1) 

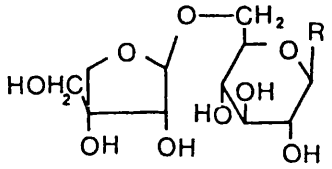
β-D-Glucopyranosides

2) 

6-O-α-L-Rhamnopyranosyl-β-D-glucopyranoside

3) 

6-O-α-L-Arabinofuranosyl-β-D-glucopyranoside

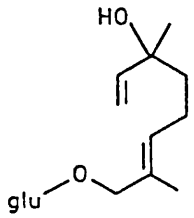
4) 

6-O-β-D-Apiofuranosyl-β-D-glucopyranoside

Fig.G.4 will be referred to again in this section during discussions of the sugar component of rhubarb glycosides.

RHUBARB GLYCOSIDES:C) ALCOHOLS

Alcohols constitute the vast majority of aglycones, and are bound to the sugar moiety *via* an ether linkage eg:-



Hydroxylinaloyl glucoside [137]

Fig.G.1 shows that alcohol aglycones can be separated into four groups: i) acetate derived, ii) isoprenoid iii) shikimate derived and iv) miscellaneous. All the tables in

this section relate to aglycones identified on the Carbowax column.

i) Acetate-derived glycosides

Table VII.1 The Acetate derived aglycones in rhubarb

ACETATE DERIVED	STALK	LEAF	ROOT
2-Methyl-3-buten-2-ol	+	+	
3-Penten-2-ol		+	
Butanol		+	
2-Methylbutanol	+		
3-Methylpentanol	+	+	
Octanal	+	+	
Hexanol	+	+	
cis-3-Hexenol		+	
2-Butoxyethanol	+		
trans-2-Hexenol		+	
Acetic acid	+	+	
Heptanol		+	
2-Ethylhexanol		+	
1-(2-Methoxypropoxy)-2-propanol		+	

ACETATE DERIVED	STALK	LEAF	ROOT
Octanol	+	+	
Butyric acid		+	
2-Methylbutyric acid		+	
2-(2-Butoxyethoxy)ethanol	+	+	
Hexanoic acid		+	
cis-3-Hexenoic acid		+	
trans-2-Hexenoic acid		+	
Octanoic acid		+	
Dodecanoic acid		+	

Straight chained alcohols: butanol, hexanol, heptanol and octanol all occur bound in rhubarb stalk and leaf (Table VII.1). The lower alcohols such as ethanol and propanol may also be present as aglycones, but coelution with the solvent made this difficult to verify. However, in their work on hog plum, Adedeji *et al.*[132] were not only able to confirm ethanol as bound but quantified it at very high levels (538ppm). Of the other alcohols bound, hexanol was identified in grape[138], pineapple[133], apricot, peach, plum[137], *Hyssopus officinalis*[120], ginger root[121], hog plum[132] and tomato[134]; hexanol and octanol in raspberry[127]; butanol, hexanol and octanol in strawberry[125] and

blackberry[55] and finally butanol, hexanol and heptanol in papaya[131]. It becomes clear from this that in most species, including rhubarb, only lower straight-chained alcohols up to C_8 are present as aglycones, with C_7 (heptanol) only rarely occurring.

The branch-chained alcohols such as 3-methylpentanol and 2-methylbutanol are much less common but have been identified in blackberry[55], papaya[131] and tomato[134]. Both of these alcohols are believed to be aglycones in rhubarb along with 2-ethylhexanol, the latter being found for the first time as an aglycone.

Unsaturated alcohols, 3-penten-2-ol, *cis*-3-hexenol and *trans*-2-hexenol were characterised as aglycones in rhubarb leaf, while 2-methyl-3-buten-2-ol occurred in the leaf and stalk. *cis*-3-Hexenol and *trans*-2-hexenol have previously been identified as bound components of strawberry[125] apricot, peach, plum[126], raspberry[127] and blackberry[55]. In their work on blackberry, Humpf and Schreier[55] drew attention to the high level [4.1 mg/kg^{-1}] of *cis*-3-hexenol present in the bound form. They pointed out that C_6 alcohols, previously thought of as catabolic products derived from linoleic/linolenic acids during cell disruption, might also be formed by anabolic pathways in which glycosides act as intermediates. This may also be the case in rhubarb leaf.

2-Methyl-3-buten-2-ol has been tentatively identified in passion fruit pulp[139] along with two other structural isomers and it was proposed that these alcohols were released from a common 3-methyl-1,3-butanediol glycoside. In rhubarb, only one isomer was detected (bound or free), suggesting that the diol glycoside was probably not a precursor.

The final group of acetate-derived aglycones in rhubarb might be termed `ether alcohols` and includes 2-butoxy ethanol, 1-(2-methoxypropoxy)-2-propanol (tent.) and 2-butoxyethoxyethanol. Only 2-butoxyethanol has been previously identified as an aglycone[133].

ii) Isoprenoid glycosides

a) Terpenes

Table VII.2 The terpene aglycones in rhubarb

TERPENES	STALK	LEAF	ROOT
1,4-Cineol	+		
Limonene	+	+	
1,8-Cineol	+		
p-Cymene	+	+	

trans-Linalool-oxide (furan)	+	+	
cis-Linalool-oxide (pyran)	+	+	
cis-Carveol	+		
Isomer of dimethyloctadienediol		+	
Isomer of dimethyloctadienediol		+	
Unknown sesquiterpene 204MW?	+		

There has been a great deal of work on terpene glycosides since their discovery in 1969[137]. Ten such glycosides were identified (some tentatively) in rhubarb (Table VII.2), which is a smaller proportion of the total number than in most other plant species. Indeed, certain common monoterpene aglycones such as linalool, nerol, geraniol and α-terpineol were absent.

1,4-Cineol, 1,8-cineol, *cis*-carveol and an unknown sesquiterpene all occurred as aglycones in rhubarb stalk. Amongst these, only carveol in celery[157] has been reported previously.

In this study, limonene and *p*-cymene have been identified as aglycones for the first time. Engel and Tressl[139] however, did note an increase in limonene concentration when the pH of passion fruit was reduced from 7 to 3 and

postulated that hydrolysed glycosidic linalool had chemically converted to limonene. A similar process may explain the presence of this non-alcoholic terpene in rhubarb.

trans-Linalool-oxide (furan) and *cis*-linalool-oxide (pyran) were identified in rhubarb stalk and leaf as aglycones. Linalool-oxide (furan) has also been found in grape[124][140][141], ginger[121], apricot[126], blackberry leaf[55] and papaya[131] whereas linalool-oxide (pyran) was present in grape[124], ginger[121], apricot and peach[126] aglycone fractions. Neither of the furan or pyran forms appeared especially favoured over the other in the glycosides of the above plants. Salles *et al.*[140] proposed three mechanisms for the presence of these linalool-oxides in aglycone fractions: i) hydrolysis of linalool-oxide glycosides, ii) hydrolysis of 3,7-dimethyloct-1-ene-3,6,7-triol and iii) oxidation of linalool during extraction. Although both ii) and iii) are possible, the recent identification of sugar bound linalool-oxides in grape[138][141] suggests that the occurrence of linalool-oxide glycoside is also likely in rhubarb.

Two dimethyloctadienediols were tentatively assigned as leaf aglycones based on M.S. data. Dimethyloctadienediols, as glycosides, have been identified in grape[124] and grape juice[141][138], ginger[121], apricot, peach, plum[126], raspberry[127], lulo fruit[128], papaya[131] and tomato[134].

Williams *et al.*[124] found higher concentrations of dienediols when grapes were treated with enzyme rather than acid and in grape juice Strauss *et al.*[138] characterised *trans*-2,6-dimethylocta-2,7-diene-1,6-diol bound to sugar moieties 1 and 3 of Fig.G.4.

In a discussion of terpene glycosides in 1989, Williams *et al.*[124] suggested that glycoside formation was the TERMINAL step of any monoterpene biosynthetic pathway. They observed that glycosides of monoterpene aglycones eventually accumulated to higher levels in grape than the free aglycones[20]. This phenomenon has also been noted in grape, apricot, mango[126][140] passion fruit[135], peach and plum[126]. In contrast, Wu *et al.*[121] noted that the ratio of monoterpene glycosides to free aglycones in ginger was much less than unity. This may be because they were analysing roots rather than fruit or vegetative tissue.

E. Stahl-Biskup[137], after observing their dual solubility in aqueous and organic environments, suggested monoterpene glycosides acted as INTERMEDIATES in the biosynthesis of monoterpenes. Others[142] proposed that glycosides could act as the vehicle between the sites of monoterpene biosynthesis and accumulation (e.g. oil gland) in plants.

Croteau and Martinkus[143] and Croteau *et al.*[144] showed that menthone is not only transported but is also catabolised as a glycoside in mint plants. In essence, it was found that

during flowering a proportion of menthone was converted to neomenthyl-β-D-glucoside, transported to the rhizome, and reoxidised to form menthone and 3,4-menthone lactone. This was then further metabolised to non-volatile polar products in the rhizome. Mint is a perennial plant in which the rhizome acts as a storage organ throughout the growing season, as well as over winter. This is also the case with rhubarb plants. The implication from this work is that although monoterpenes are transported and cycled as glycosides, they are not stored (long-term) as such. The small number of rhubarb root glycosides, only seven to date, would also seem to support this mechanism.

b) Norisoprenoids

There has been a great deal of interest in norisoprenoid glycosides as possible precursors of extremely low threshold flavour compounds such as vitispiranes, theaspiranes and edulans. In addition, many of the bound norisoprenoids, when released during hydrolysis, have a significant flavour impact in their own right and these factors combined made it of interest to investigate these glycosides in rhubarb.

Unfortunately, due to the non-availability of standard compounds, the analysis of these components was difficult. Therefore, many of those present as aglycones in rhubarb were tentatively assigned from mass spectral data and

approximate retention times only. These norisoprenoids are shown in Table VII.3.

Table VII.3 The norisoprenoid aglycones in rhubarb

NORISOPRENOIDS	STALK	LEAF	ROOT
Unknown 192MW?		+	
Unknown 192MW?		+	
2,2,6,8-Tetramethyl-7,11-dioxatricyclo[6.2.1.0]undec-4-ene		+	
1,2-Dihydro-1,1,6-trimethyl-naphthalene		+	
Dehydroionone 190MW?		+	
Unknown 196MW?		+	
Isomer of trimethylphenyl-2-butanone		+	
Unknown 192MW?		+	
Unknown. base peaks m/z 123, 163	+	+	
3,4-Didehydro-β-ionol		+	
Unknown 210MW?		+	
Unknown 208MW?		+	

NORISOPRENOIDS	STALK	LEAF	ROOT
Isomer of dihydroxyionone		+	
Unknown 206MW?		+	
3-Oxo-α-ionol	+	+	
4-Oxo-β-ionol	+	+	
3-Hydroxy-7,8-dihydro-β-ionol		+	
4-Oxo-7,8-dihydro-β-ionol	+	+	
3-Hydroxy-β-ionone	+	+	
3-Hydroxy-5,6-epoxy-β-ionone	+	+	

4-Oxo-β-ionol, although identified in rhubarb stalk, was the only norisoprenoid in rhubarb tissue not found as an aglycone in the leaf. This volatile is also known to occur in this form in raspberry[127] and passion fruit[145]. 3-Oxo-α-ionol is a common aglycone in plants and there is evidence for its presence in rhubarb as well as in quince[146], apricot, peach[126], raspberry[127], blackberry[55], lulo fruit[128], mango[130], papaya[131] tomato[134], passion fruit[145] and sloe tree leaf[123]. The glycoside of 3-oxo-α-ionol has been shown not to hydrolyse at the pH found in rhubarb stalks (3.2)[147] [145] and, along with the glycoside of vomifoliol, is proposed as having a role in biological functions such as stomatal closing[147]. It is notable that 3-oxo-α-ionol, along with dehydrovomifoliol, were the only

α-norisoprenoids identified in rhubarb. This was in common with most other plants where β-norisoprenoids greatly outnumber α-norisoprenoids as aglycones.

3,4-Didehydro-β-ionol, a component of the rhubarb leaf aglycone fraction, has also been identified in tomato[129]. Although this ionol is known to form when 3-hydroxy-β-ionol aglycone is heated at pH 3.2[146], this is unlikely to occur during the less severe conditions of this analysis.

3-Hydroxy-7,8-dihydro-β-ionol has been identified as an aglycone in quince[146], strawberry[125], apricot, peach and plum[126]. In rhubarb it was exclusively present in the leaf, a result which compares with recent aglycone analysis in blackberry and sloe leaves[55][129] where 3-hydroxy-7,8-dihydro-β-ionol occurred at especially high levels (7.3 and 3.9 mg/kg).

4-Oxo-7,8-dihydro-β-ionol and 3-hydroxy-β-ionone were both bound components in rhubarb stalks and leaves. Quince[146], apricot, peach, plum[126], raspberry[127], blackberry leaf[55] and passion fruit[134] have also been found to contain at least one of these aglycones.

3-Hydroxy-5,6-epoxy-β-ionone is a major norisoprenoid aglycone in rhubarb leaf and stalk that is not widely found in other plants. It has been identified in apricot[126], *Epimedium grandiflorum var thunbergianum*[148], raspberry[127]

and lulo fruit[128]. In the *Epimedium spc.* 3-hydroxy-5,6-epoxy-β-ionone was detected bound as a β-D-glucopyranoside [1 in Fig.G.4].

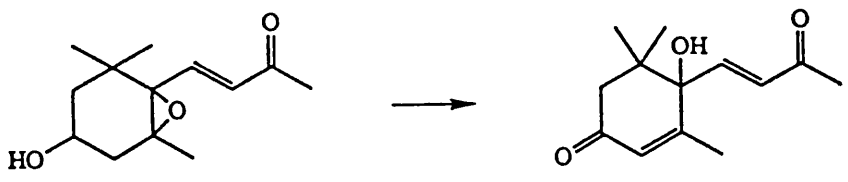
Norisoprenoid artifact formation

In this analysis the presence of norisoprenoid glycosides in rhubarb was proven by hydrolysis followed by the subsequent GC/MS detection of the aglycones released. The lability of some norisoprenoids, coupled to the conditions required to release them as aglycones, meant that it was possible that both oxidatively and thermally derived artifacts might form. Both oxidative and thermal artifacts will now be discussed in turn:-

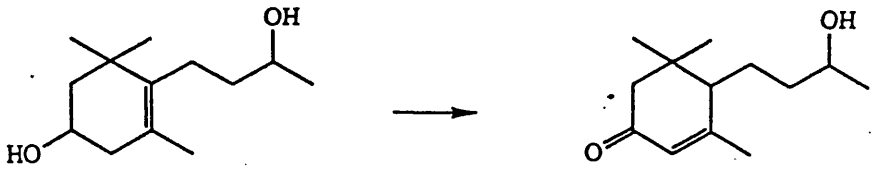
Oxidative artifacts *via* fungal glycosidases.

Recently, Sefton and Williams[149] have shown that the presence of high levels of fungal derived enzymes causes oxidation of some norisoprenoid aglycones (Fig.G.5).

Fig.G.5 Fungal glycosidase catalysed oxidation of norisoprenoids



3-Hydroxy-5,6-epoxy-β-ionone Dehydrovomifoliol



3-Hydroxy-7,8-dihydro-β-ionol 3-Oxo-7,8-dihydro-α-ionol

Plant derived enzymes do not act in this way. It was found that when greater than 20mg of fungal enzyme was added to glycosides from 250g of grape juice some or all of the two norisoprenoids above were oxidised. In rhubarb only 3mg of fungal enzyme was added to the glycosides derived from 1000g of plant material. This level makes it unlikely that the oxidation shown in Fig.G.5 occurred and suggests that the dehydrovomifoliol identified in rhubarb leaf (DB-5 column) was indeed present as an aglycone.

Thermally derived artifacts from norisoprenoids

There is evidence of several thermal breakdown products in the rhubarb extract released from glycosides. Four norisoprenoid aglycones, lacking hydroxy groups, were tentatively identified in rhubarb leaf: 2,2,6,8-tetramethyl-7,11-dioxatricyclo [6.2.1.0] undec-4-ene (Riesling acetal),1,2-dihydro-1,1,6-trimethylnaphthalene (TDN), a dehydroionone and a trimethylphenyl-2-butanone isomer. These compounds have also been noted as thermal

breakdown products from labile precursors in grapes[152], wine[151], redcurrant leaves[122], sloe-tree leaves[123] and in passion fruit[145]. There are three aglycone precursors which could produce these norisoprenoid artifacts:-

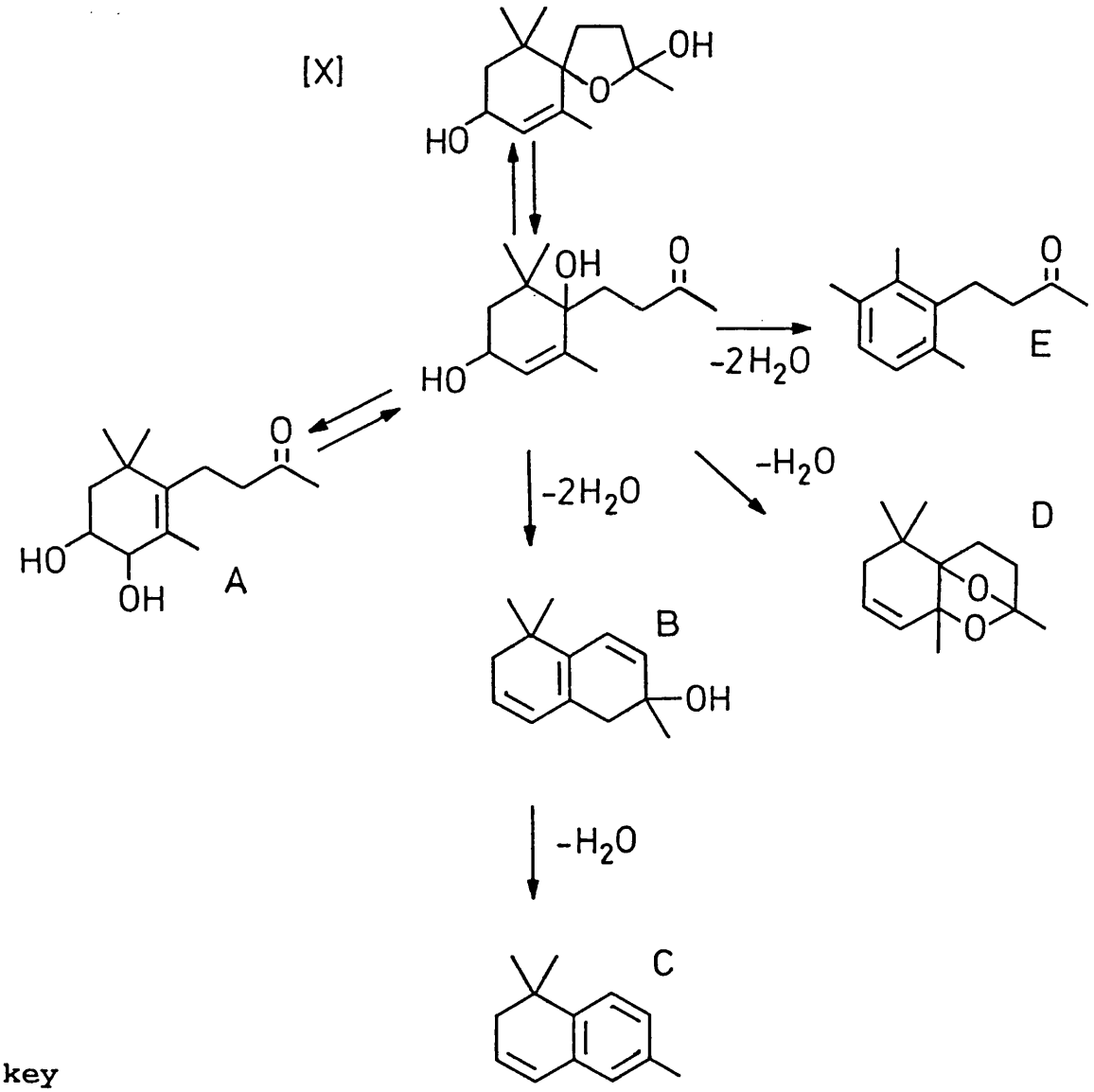
1) 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol
2) Megastigmadienetriols
3) 3-Hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene

Each mechanism of formation will be considered in turn:-

1) The artifacts formed from 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol

Fig.G.6 shows how 2,6,10,10-tetramethyl-1-oxaspiro [4.5]dec-6-ene-2,8-diol (X), when heated in an acidic medium, can fragment into a number of derivatives, all of which might be mistakenly regarded as the actual aglycone. Of these breakdown products, A is too polar and B too unstable[151] to elute under the conditions of GC-MS analysis. However, C was identified in rhubarb leaf, and peak 31 (DB-WAX) of this extract, with major fragments at m/z 43, 125, 138, was tentatively assigned as D. The elution time of the peak also corresponds with that of this compound. E was another component present in the rhubarb leaf aglycone fraction.

Fig.G.6 Breakdown products of 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol [X]



key

A 3,4-Dihydroxy-7,8-dihydro-β-ionone

B 6-Hydroxy-1,1,6-trimethyl-1,2,5,6-tetra hydronaphthalene

C 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)

D 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0]undec-4-ene

E 4-(2,3,6-Trimethylphenyl)-2-butanone

Humpf *et al.*[122] observed that acid hydrolysis of the glycoside of 3,4-dihydroxy-7,8-dihydro-β-ionone (A) also produced breakdown products B - E. This suggests that there is a reversible interconversion of X and A [see Fig.G.6] and that in rhubarb leaf either could be present as a glycoside.

2) The artifacts formed from megastigmadienetriol

During investigation of sloe leaves[123] and of grapes[152], two dienetriols were found to hydrolyse easily:- megastigma-5,7-diene-3,4,9-triol and megastigma-4,7-diene-3,6,9-triol.

The breakdown products were similar to those found in rhubarb and detailed in Fig.G.6 but with the addition of other artifacts such as actinidols and isophorones. Although these dienetriols may be aglycones in rhubarb, their poor volatility means they are not amenable to GC/MS analysis.

3) The artifacts formed from 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene

1,2-Dihydro-1,1,6-trimethylnaphthalene (TDN) has been found in excessively heated passion fruit juice. Winterhalter[145] proposed that it formed from 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene, bound as a glycoside, and

confirmed its presence by GC/MS. However, the conditions employed in the present work did not involve excessive heating, suggesting that 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene was neither an aglycone, nor a precursor of the TDN found in rhubarb.

Clearly GC/MS analysis of extractives is greatly complicated when thermally labile compounds are believed to be present. This would appear to be especially relevant when norisoprenoid aglycones are being considered. However, this does indicate how a complex array of norisoprenoids and norisoprenoid derivatives may develop in rhubarb, greatly enriching its flavour.

iii) Shikimate-derived glycosides

Phenol, benzyl alcohol and 2-phenylethanol are ubiquitous aglycones in the plant world and not surprisingly were identified in rhubarb stalk and leaf. Williams *et al.*[135][140] identified benzyl alcohol and 2-phenylethanol in grapes bound as β-D-glucopyranosides, β-rutinosides and 6-O-α-arabinofuranosyl-β-D-glucopyranosides [structures 1,2 and 3 in Fig.G.4]. Although phenols appear as aglycones in all plant materials, few workers have discussed their possible contribution to flavour.

Table VII.4 The shikimate-derived aglycones in rhubarb

SHIKIMATE DERIVED	STALK	LEAF	ROOT
1,3-Dimethylbenzene		+	
Benzaldehyde		+	+
Naphthalene	+		
Guaiacol		+	
Benzyl alcohol	+	+	
2-Phenylethanol	+	+	
o-Cresol	+		
Phenol	+	+	
Isomer of dimethylnaphthalene	+	+	
Anisaldehyde	+	+	+
p-Cresol	+	+	
Isomer of trimethylnaphthalene		+	
Eugenol	+	+	
4-Vinylguaiacol	+	+	
Isomer of dimethoxyphenol	+		
2,6-Dimethoxyphenol	+	+	
Isomer of dimethoxyphenol	+		
Cinnamyl alcohol	+		

SHIKIMATE DERIVED	STALK	LEAF	ROOT
Methyl 4-hydroxybenzoate	+		
4-Propenylphenol	+	+	
4-Vinylphenol	+	+	
Benzoic acid	+	+	
Phenylacetic acid	+	+	
3,4,5-Trimethoxybenzaldehyde	+	+	
4-Allyl-2,6-dimethoxyphenol	+	+	+
Vanillin	+	+	+
2,6-Dimethoxy-4-vinylphenol	+	+	
Methyl vanillate	+		+
Acetovanillone	+	+	
Zingerone	+	+	
Frambinone	+	+	

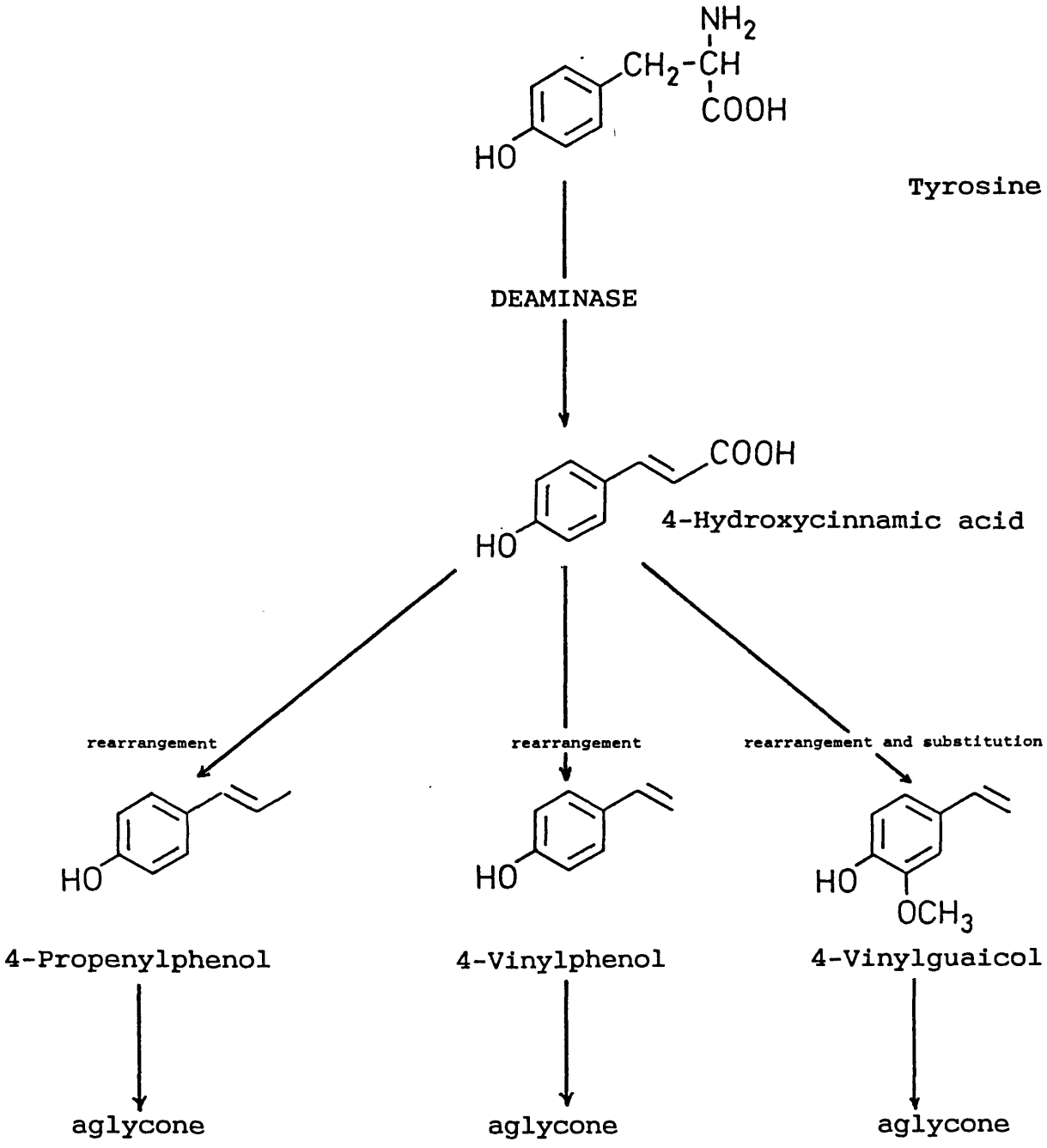
Williams *et al.*[124] considered the shikimate-derived group of volatiles to be highly significant in the flavour of grape juice and characterised twenty-seven such compounds present as aglycones. Since shikimate-derived metabolites constitute by far the largest group of aglycones [25 out of 53 in the stalk], their contribution to the flavour of rhubarb may be considerable.

It is notable that although longer-chained and multi-substituted compounds are very common extractives of plants, the relatively simple phenols such as cresol, guaiacol and dimethoxyphenol, are infrequently found. For example *p* and *o*-cresols were aglycones in raspberry[127] and papaya[131] respectively, guaiacol was found in blackberry[55] and lulo fruit[128] while dimethoxyphenol has been identified only once, in an aglycone fraction from grape[124]. In contrast, rhubarb stalk contains two isomers of cresol and three of dimethoxyphenol bound as glycosides, while in the leaf, guaiacol as well as one isomer each of cresol and dimethoxyphenol were detected. Clearly rhubarb differs from other plant species in containing high numbers of short chained, methoxy-substituted phenols as aglycones and this might be indicative of a biosynthetic pathway peculiar to *Rheum rharbarbarium*.

Slightly longer side-chained phenols such as 4-propenyl phenol, 4-vinylphenol and 4-vinylguaiacol were identified as aglycones in rhubarb stalk and leaf. They are also known components of strawberry[125], apricot, peach, plum[126], raspberry[127], blackberry fruit and leaf[55], lulo fruit[128] and tomato[129]. The regularity of substitution at the 4-position is a consequence of their derivation from the shikimic acid biosynthetic pathway *via* tyrosine [Fig.G.7].

Fig.G.7

The Formation of volatile phenols from Tyrosine



2-Methoxy-4-allylphenol (eugenol) and 2,6-dimethoxy-4-vinyl phenol were identified as aglycones in rhubarb stalk and leaf but not the root, whereas 4-allyl-2,6-dimethoxyphenol

was present in all three. Whilst eugenol has been recorded in many plants[127] and 4-allyl-2,6-dimethoxyphenol is in part bound as a glycoside in pineapple[133], 2,6-dimethoxy-4-vinylphenol does not appear to have been found previously as an aglycone.

Vanillin, zingerone and frambinone, all important flavour volatiles, were identified as aglycones in rhubarb stalk and leaf. Vanillin has also been identified in grape[124], strawberry[125], apricot, peach and plum[126], raspberry[127], *Hyssopus officinalis*[120] and tomato[134], while zingerone occurred in grape[124][138] and raspberry[127] and frambinone in peach[126] and raspberry[127]. It is notable that of these three aglycones only vanillin was detected in rhubarb root whilst in a similar analysis of Chinese rhubarb root [See Chapter 1], none were found in the edible varieties investigated.

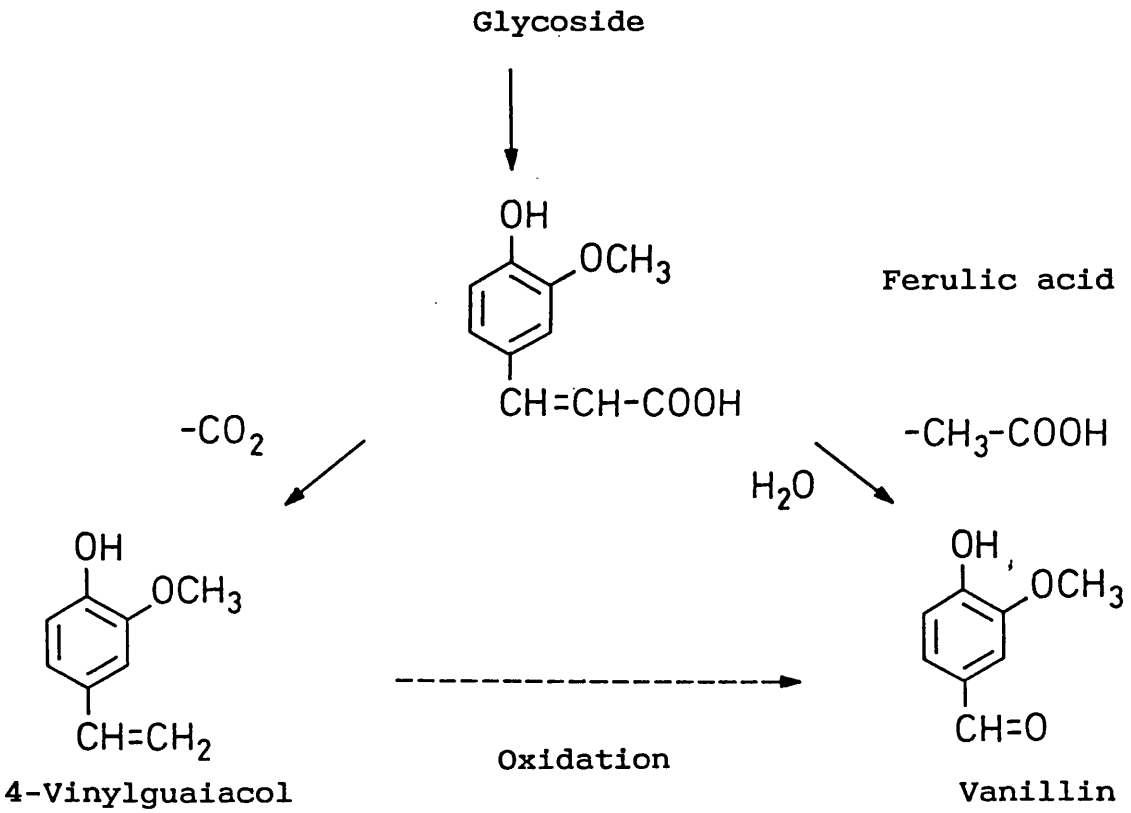
As mentioned earlier, phenolic aglycones derive from aromatic amino acids via shikimic acid pathway metabolites such as *p*-coumaric, caffeic and ferulic acids. These metabolites are themselves present in many plants in the form of glycosides, very commonly as glucose esters. These acids are insufficiently volatile to be identified by the GC/MS analysis used in this work. Marlatt *et al.*[134] analysed the phenolic aglycones in tomatoes, methylating the nonvolatile acids to enable identification by GC/MS. In this way, the above three phenolic acids, along with

sinapic acid were elucidated. It is probable that similar glycosides are present in rhubarb. It has been noted that there is little metabolic turnover of such glycosides in living cells and it is proposed that they function as a storage mechanism for toxic phenols[26]. This contrasts with the rapid synthesis and turnover of free phenolic compounds in the shikimic acid pathway.

At present only benzyl alcohol and 2-phenylethanol glycosides have been characterised intact. This raises the possibility that at least some of the other phenolic aglycones in Table VII.4 may be artifacts. Buttery *et al.*[129] in comparing acid and enzymatically hydrolysed tomato glycosides found that only acid treatment released 4-vinylphenol and 4-vinylguaiacol. It was hypothesised that these compounds were formed by decarboxylation of coumaric and ferulic acids and that it was these acids which were present as glycosides. Recent work by Peleg *et al.*[48] on the storage of model solutions of orange juice containing ferulic acid supported this hypothesis. They proposed a pathway for the degradation of ferulic acid glycosides [Fig.G.8].

Fig.G.8

Mechanism for the degradation of ferulic acid glycoside in citrus juice



4-Vinylguaiacol was a significant product at 25°C/pH 3.8 after 7 days, whereas vanillin formed after 7 days at 35°C/ pH 3.8. A similar mechanism can be proposed for 4-vinylphenol from coumaric acid. While enzymatic hydrolysis of aglycones in rhubarb [pH 5.7/40°C, 2 days] did not exactly replicate these conditions, artifact formation cannot be ruled out.

Another source of artifacts may be *via* thermal decomposition of phenolic acids in the GC injector. Adedeji *et al.*[134] showed that ferulic acid was, to a degree, soluble in pentane/ether and so was transferable to the GC injector. It is probable that similar solvation and transference occurred in the rhubarb analysis. However, unlike in the above analysis, the injector was only heated to 200-220°C (DB-WAX column). This may have allowed poorly volatile ferulic acid to remain in the injector, forming 4-vinylguaiacol and vanillin by decarboxylation and oxidation.

IV) Miscellaneous

Indole was positively identified at trace levels as an aglycone in rhubarb stalk. It has also been identified as an aglycone in tomato[129].

Conclusion: Rhubarb Glycosides

From the above discussion it can be seen that glycosides are key components in many plant species. Several theories have been proposed for their role in plants: a) aglycone storage, b) detoxification, c) terminal step of aglycone biosynthesis, d) aglycone transport, e) biosynthetic intermediary and f) biological activity.

Characterisation of glycosides by Park *et al.*[153] has suggested that daily changes of their levels in grapes may be triggered by environmental temperature fluctuation, the general trend being that glycoside concentration drops with increased temperature. A similar effect may occur in rhubarb and research into such daily fluctuations may help to elucidate the role a) - f) of its glycosides. Consideration of such changes could help to maximise the level of glycosides prior to commercial processing. This is of importance as it becomes clear that glycosides are a potential source of flavour chemicals in rhubarb.

Williams *et al.*[124] studying enzymically hydrolysed grape glycosides and those released at 50°C/pH 3.2 found that sensory assessment demonstrated that more flavour volatiles were released at pH 3.2 than by glycosidases. As the natural pH of rhubarb stalk is 3-3.5 it is probable that glycosides could be hydrolysed during processing, particularly when heat is applied. This may explain why

cooked rhubarb is favoured culinarily as the release of volatiles such as norisoprenoids, frambinone etc., and their acid rearrangement products, may improve the flavour.

CHAPTER 8

GENERAL CONCLUSION

This study has described the flavour chemistry of fresh and processed rhubarb and investigated the presence of glycosides in all parts of the plant. The enzymatic and chemical formation of these volatiles has been discussed and has been related to the flavour characteristic and the commercial potential of rhubarb extracts.

Future work could correlate the presence of each volatile with its ultimate impact on the flavour of rhubarb. Such work would involve organoleptic trials, aroma dilution experiments and 'off G.C.' sniffer port analysis. In this way, the volatile formation mechanisms described in this study could be priorised and this knowledge used to increase the flavour of rhubarb extracts to a maximum.

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